PDX-1 MODULATES ENDOPLASMIC RETICULUM CALCIUM HOMEOSTASIS IN THE ISLET β CELL VIA TRANSCRIPTIONAL ENHANCEMENT OF SERCA2B

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Dedication

This dissertation is dedicated to my wife, Amy, who has supported me through every triumph and every adversity.

“A loving heart is the beginning of all knowledge.” – Thomas Carlyle

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Justin Sean Johnson

PDX-1 MODULATES ENDOPLASMIC RETICULUM CALCIUM HOMEOSTASIS IN THE ISLET β CELL VIA TRANSCRIPTIONAL ENHANCEMENT OF SERCA2B

Diabetes mellitus (DM) affects an estimated 285 million people worldwide, and a central component of diabetes pathophysiology is diminished pancreatic islet β cell function resulting in the inability to manage blood glucose effectively. The β cell is a highly specialized metabolic factory that possesses a number of specialized characteristics, chief among these a highly developed endoplasmic reticulum (ER). The sarco endoplasmic reticulum Ca^{2+} ATPase 2b (SERCA2b) pump maintains a steep Ca^{2+} gradient between the cytosol and ER lumen, and while the Pancreatic and duodenal homeobox 1 (Pdx-1) transcription factor is known to play an indispensable role in β cell development and function, recent data also implicate Pdx-1 in the maintenance of ER health. Our data demonstrates that a decrease of β cell Pdx-1 occurs in parallel with decreased SERCA2b expression in models of diabetes, while *in silico* analysis of the SERCA2b promoter revealed multiple putative Pdx-1 binding sites. We hypothesized that Pdx-1 loss under inflammatory and diabetic conditions leads to decreased SERCA2b with concomitant alterations in ER health.

To test this, siRNA-mediated knockdown of Pdx-1 was performed in INS-1 cells. Results revealed reduced SERCA2b expression and decreased ER Ca^{2+}, which was measured using an ER-targeted D4ER adenovirus and fluorescence lifetime imaging microscopy. Co-transfection of human Pdx-1 with a reporter fused to the human *SERCA2* promoter increased luciferase activity three-fold relative to the empty vector control, and direct binding of Pdx-1 to the proximal *SERCA2* promoter was confirmed by chromatin immunoprecipitation. To determine whether restoration of SERCA2b could

rescue ER stress induced by Pdx-1 loss, $Pdx1^{+/-}$ mice were fed high fat diet for 8 weeks. Isolated islets from these mice demonstrated increased expression of spliced Xbp1, signifying ER stress, while subsequent SERCA2b overexpression in isolated islets reduced spliced Xbp1 levels to that of wild-type controls. These results identify SERCA2b as a direct transcriptional target of Pdx-1 and define a novel role for altered ER Ca^{2+} regulation in Pdx-1 deficient states.

Future studies will investigate the interaction of Pdx-1 with other transcription factors at the *SERCA2* promoter, particularly peroxisome proliferator-activated receptor gamma and forkhead box protein O1.

Carmella Evans-Molina, M.D., Ph.D., Chair

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List of Abbreviations

ARC	Apoptosis Repressor with Caspase Recruitment Domain
Atf4	Activating Transcription Factor 4
Atf6	Activating Transcription Factor 6
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
Bcl2	B-cell Lymphoma protein 2
BiP	Binding Immunoglobulin Protein
bFGF	Basic Fibroblast Growth Factor
BG	Blood Glucose
Ca^{2+}	Calcium ion
CAMK	Ca^{2+}/calmodulin-Dependent Protein Kinase
CAMKII	Ca^{2+}/calmodulin-Dependent Protein Kinase 2
CBP	Creb-Binding Protein
C/EBP	CCAAT-Enhancer Binding Protein
ChIP	Chromatin Immunoprecipitation
CHOP	C/EBP Homology Protein
Co-IP	Co-immunoprecipitation
D4ER	Endoplasmic Reticulum Targeted Vitamin D4 Receptor Peptide
DM	Diabetes Mellitus
DMEM	Dulbecco's Modified Essential Medium
DTT	Dithiothreitol
ECFP	Enhanced Cyan Fluorescent Protein
EGF	Epidermal Growth Factor
EGTA	Ethylene Glycol Tetraacetic Acid

ER	Endoplasmic Reticulum
ERAD	Endoplasmic Reticulum-Associated Degradation
FFA	Free Fatty Acid
FLIM	Fluorescent Lifetime Microscopy
FOXO1	Forkhead box Protein O1
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GCK	Glucokinase
GDM	Gestational Diabetes Mellitus
GH	Growth Hormone
GLP-1	Glucagon-like Peptide 1
GLUT1	Glucose Transporter 1
GLUT2	Glucose Transporter 2
GLUT2	Glucose Transporter 2 gene
GRP-58	ER protein 57
GSIS	Glucose Stimulated Insulin Secretion
GTT	Glucose Tolerance Test
HbA1C	Hemoglobin A1C
HB-EGF	Heparin-Binding EGF-like Growth Factor
HFD	High Fat Diet
HG	High Glucose
HG+IL-1b	High Glucose and Interleukin 1-beta (*in vitro* model of DM)
HNF-1α	hepatic nucleocyte factor 1 alpha
HNF-3β	hepatic nucleocyte factor 3 beta
IGF-1	Insulin-like Growth Factor 1
IL-1β	Interleukin 1-beta
INS-1	Cultured Rat Insulinoma Cell Line INS-1

INS1	Insulin Gene 1 (mouse)
INS2	Insulin Gene 2 (mouse)
IPGTT	Intraperitoneal Glucose Tolerance Test
IRS1	Insulin Receptor Substrate 1 gene
JNK	c-Jun N-terminal Kinase
KATP	Potassium-linked ATP channels
KSIS	Potassium stimulated Insulin Secretion
LIRKO	Liver Insulin Receptor Knockout (mouse model)
Mafa	v-maf Avian Musculoaponeurotic Fibroscarcoma Oncogene Homolog A
MAPK	Mitogen-Activated Protein Kinase
MIN-6	Cultured Mouse Insulinoma 6 Cell Line
mRNA	Messenger Ribonucleic Acid
miRNA	Micro Ribonucleic Acid
MODY	Maturity-Onset Diabetes of the Young
NC	Normal Chow
NDM	Neonatal Diabetes Mellitus
NIH-3T3	Cultured Mouse Fibroblast Cell Line NIH-3T3
Nkx2.2	NK2 Homeobox Protein 2
Nkx6.1	NK6 Homeobox Protein 1
NFκB	Nuclear Factor kappa-Light-Chain-Enhancer of Activated B Cells
n.s.	Not (statistically) Significant
ns	Nanoseconds
OGTT	Oral Glucose Tolerance Test
p38	p38 MAP Kinase
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction

PDGF	Plate-Derived Growth Factor
Pdx-1	Pancreatic and Duodenal Homeobox protein 1
PERK	Protein Kinase RNA-like Endoplasmic Reticulum Kinase
PI3-K	Phosphoinositide 3-Kinase
PKC	Protein Kinase C
PKCζ	Atypical Protein Kinase C zeta
PKG	Protein Kinase G or Cyclic-GMP-Dependent Protein Kinase
PPARγ	Peroxisome Proliferator-Activated Receptor γ (Gamma)
$PPAR\gamma_{S273D}$	PPARγ Serine-to-Aspartate Mutant at the 273rd Amino Acid Residue
PPRE	PPAR Response Element
PUFA	Poly-Unsaturated Fatty Acid
RBG	Random Blood Glucose Measurement
ROS	Reactive Oxygen Species
RXR	Retinoid X Receptor
SAPK2	Stress-Activated Protein Kinase 2
SEM	Standard Error of the Mean
SERCA2	Sarco-endoplasmic Reticulum Calcium ATPase
SET7/9	SET-Domain Containing Histone-Lysine N-Methyltransferases 7 and 9
siRNA	Small Interfering RNA
SP1	Transcription Factor Specificity Protein 1
SREBP	Sterol Regulatory Element Binding Protein
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
T3	Triiodothyronine
TFAM	Nuclear-Encoded Mitochondrial Factor A
TG	Thapsigargin

TGFβ	Transforming Growth Factor beta
TNFα	Tumor Necrosis Factor alpha
TRPV1+	Transient Receptor Potential Vanilloid Subfamily Member 1 Positive
TZD	Thiazolidinedione
UPR	Unfolded Protein Response
USF	Upstream Stimulatory Factor
VSMC	Vascular Smooth Muscle Cells
Wfs1	Wolframin
Xbp1	X-box Binding Protein 1

Chapter I. Introduction

I.A. Diabetes Mellitus

Diabetes mellitus (DM) is a metabolic disorder characterized by chronically elevated blood glucose due to a relative or complete deficiency of insulin [1]. Insulin is a peptide hormone synthesized and released by the β cells of the pancreas, and its physiological effects include stimulation of glucose uptake in peripheral tissues, inhibition of hepatic gluconeogenesis, and growth stimulation [2]. Diabetes mellitus is divided into two major types designated as Type 1 diabetes mellitus (T1DM), which is characterized by autoimmune etiology, insulin dependence, and potentially life-threatening symptoms at diagnosis and Type 2 diabetes mellitus (T2DM), which is characterized by hyperinsulinemia and is correlated with increased age and body mass index [3]. Chronic hyperglycemia in both forms of DM leads to osmotic imbalance, microvascular damage, peripheral nerve impairment and potentially lethal effects on the cardiovascular and renal systems [1, 4, 5].

In 2010 DM was the 7th leading cause of mortality in the United States (**Figure 1**), and it also contributed to seven of the remaining top ten causes [5]. Over 25 million Americans were estimated to be affected by diabetes mellitus in 2011 with an approximate net economic cost exceeding $174 billion per year in the United States alone [1]. According to the World Health Organization, by the year 2025 there will be more than 380 million people worldwide living with DM, while an additional 418 million people will have impaired glucose tolerance or pre-diabetes [6]. Understanding the pathophysiology of DM in order to design better strategies for treatment and prevention is crucial to public health in the United States and worldwide.

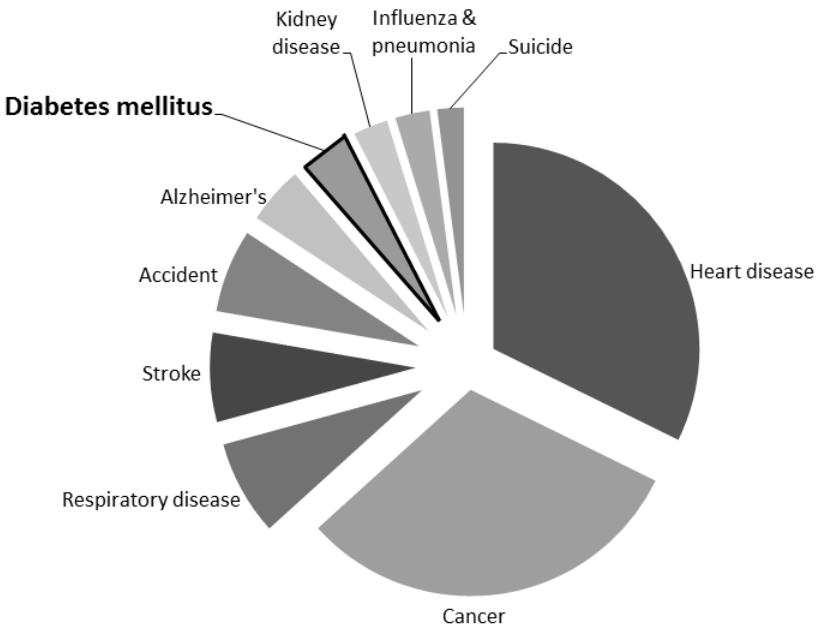


Figure 1. Ten Most Common Causes of Mortality in the United States, 2010.

Diabetes mellitus was the 7th leading cause of death in the United States in the year 2010. DM also has an established link to heart disease, cancer, stroke, Alzheimer's disease, and kidney disease. Recent research suggests that DM has a link to respiratory disease and pneumonia as well.

I.A.1. History of Diabetes Mellitus

The first recorded reference to a syndrome including excess urination was by ancient Egyptians in approximately 1500 BCE., but polyuric syndromes were first called "diabetes" by the 1st century Greek physician Araetus of Cappodocia [7]. The first diabetes cases recorded occurred in children and adults who were not observed to be overweight which suggests an autoimmune etiology, but cases of diabetes in adults with above average body fat were also recorded [7, 8]. Prior to the 20th century, there were no effective treatments for diabetes.

Although physicians could readily diagnose and differentiate DM from other polyuric syndromes, the etiology of DM was not understood until the 19th century. Prior to that time proposed causes were manifold, including emotional excitability, temperature changes, and socially unacceptable behaviors [7]. The discovery by French biochemist and physiologist Claude Bernard that the liver was capable of synthesizing carbohydrates was the first substantiation of the liver's role in DM [9]. Prior to Bernard's discovery of glycogen it had been thought that carbohydrates were of exclusively vegetable origin [10], and therefore the understanding that animals were capable of glucose synthesis was a significant conceptual advance relevant to DM.

The discovery that provided the first evidence for the pancreatic role in DM was the observation of a dog's repeated urination on a laboratory floor. The animal had been recently pancreatectomized, and when physiologist Oskar Minkowski tested the dog's urine he found that it contained a high concentration of glucose. Repeated experiments involving pancreatectomy confirmed the finding of glycosuria, for the first time linking the pancreas with a pathognomonic symptom of diabetes mellitus [7].

After Minkowski's discovery of the link between DM and the pancreas became widely known, physiologists sought to understand how the pancreas controlled glucose levels in the blood. Investigators at the University of Toronto including Fredrick Banting, Charles

Best, James J. R. MacLeod, and James B. Collip hypothesized that an extract of animal pancreas could be given to diabetic persons to control blood glucose, and they directed their efforts towards isolating and purifying this unknown substance. Eventually Banting, Best, MacLeod and Collip developed a protocol to isolate a pancreatic extract they named “insulin” which reduced blood glucose when injected into pancreatectomized dogs. Isolated animal insulin was subsequently found to lower blood glucose in diabetic humans [11, 12]. Refinement of the insulin isolation process soon led to what was considered at the time to be a “miraculous” reversal of this fatal disease, and the modern era of insulin treatment for DM began in the year 1922 [13].

I.A.2. Classification of Diabetes Mellitus

Diabetes mellitus is divided into two major types as well as other less prevalent types. The two largest categories of diabetes mellitus are Type 1 (T1DM) and Type 2 (T2DM). T2DM is most prevalent in the United States, comprising approximately 90-95% of DM cases with T1DM making up the majority of the remaining 5-10% [1, 14]. Types of diabetes mellitus not categorized as either Type 1 or Type 2 include Gestational diabetes mellitus (GDM), which occurs in pregnancy [15], Neonatal diabetes mellitus (NDM) in infants [16], single-gene mutations causing Maturity Onset Diabetes of the Young (MODY) [17], as well as other less prevalent forms including: monogenic and polygenic congenital DM syndromes, “bronze diabetes” due to hemochromatosis, cystic fibrosis related DM, or DM due to additional factors including drug or steroid use or abuse, trauma of the exocrine pancreas, β cell mitochondrial disorders, and defects in the insulin receptor [3, 18, 19]. Despite increased understanding of the mechanisms of DM as well as multiple potential treatments available, the incidence of both T1DM and T2DM are increasing each year throughout the world [4, 20].

T1DM is caused by autoimmune-mediated destruction of the β cell population in the pancreatic islets of Langerhans [3, 4, 21], and is characterized by nearly complete loss of insulin secretion [4]. T1DM differs from T2DM primarily by the symptom of hypoinsulinemia, although individuals with advanced T2DM may also lack the ability to secrete adequate insulin [22]. T1DM may have a rapid onset of only a few months, or residual β cell function may remain for years or decades prior to the necessity for exogenous insulin supplementation [3]. Polygenic heritability factors including HLA-subtype can be used to predict T1DM in certain sub-populations, with detection of T1DM primarily occurring via serum testing for syndrome-specific auto-antibodies. T2DM results from a combination of insulin resistance at the level of the adipose, skeletal muscle, and liver, impaired gluconeogenesis and insufficient insulin secretion from the endocrine pancreas [23, 24].

In obese individuals the prodrome of pre-diabetes includes a state of chronic insulin resistance in peripheral tissues [25-28]. The first stage of insulin resistance was originally observed to be due to nutrient surfeit primarily affecting adipocytes, hepatocytes and skeletal muscle myocytes [29]. As measured serum levels of free fatty acids increased an initial compensatory hyperinsulinemia resulted. When insulin hypersecretion compensation fails to suppress serum free fatty acid levels, susceptible individuals progress to fasting hyperglycemia and T2DM [30]. After these initial observations, a previously unappreciated aspect of T2DM was that in obesity systemic inflammation also contributes to insulin resistance [29]. This adipose tissue inflammation bears a strong correlation with obesity, but not all persons with obesity-associated inflammation become diabetic [31]. Thus it was determined that obesity and inflammation contribute to insulin resistance, and that insulin resistance plays a role in the onset of T2DM, yet insulin resistance is not the sole causative factor [29, 32-38].

I.A.3. Diagnosis and Treatment of Diabetes Mellitus

The symptoms of untreated T1DM and T2DM are quite different, although they do share some common features. Symptoms of T1DM are generally more obvious than T2DM and often include excessive urination (polyuria), excessive thirst (polydipsia), excessive hunger (polyphagia), unexplained weight loss, a “fruity” odor on the breath, and diabetic ketoacidosis – which can cause coma, seizure, and death [4, 39]. In contrast, persons with T2DM may display no overt symptoms initially [1], but when they do experience symptoms excessive urination (polyuria), excessive thirst (polydipsia), fatigue (anergy), and depression [40] are the most common complaints. Because T2DM symptoms can be subtle or may be attributed to other etiologies by healthcare practitioners, those with T2DM are often diagnosed due to health screening rather than by symptomology [39].

The American Diabetes Association, in conjunction with other international health organizations, has defined the following criteria for diagnosis of either T1DM or T2DM: a hemoglobin A1C (HbA_{1C}) value of >6.5%, fasting plasma glucose concentration $\geq$126 mg/dL, plasma glucose concentration >200 mg/dL two hours after intake of 75 g oral glucose solution (oral glucose tolerance test, or OGTT), or random blood glucose concentration (RBG) >200 mg/dL in a person with other hyperglycemic symptoms. Repeat testing is preferable if the individual does not display excessive hyperglycemia or overt diabetic symptoms at the time of testing [39].

The two most prevalent types of DM have different etiologies and require different standards of care. Treatment of T1DM focuses on replacing insulin, while initial treatment of T2DM focuses on improving insulin sensitivity and preserving functional β cell mass [39].

Prior to the advent of exogenous insulin supplementation, the threshold for successful treatment of diabetes was considered to be the cessation of excess sugar

excretion in the urine [10]. It is now known that glucose is not detectable in the urine below a blood glucose level of approximately 200 mg/dL, but health outcomes are improved if blood glucose levels are maintained at significantly lower levels. Although blood glucose targets should be adjusted by a physician on an individual basis, for most diabetic individuals the recommended targets include: hemoglobin A1c (HbA1C) level of less than 7%, preprandial capillary glucose concentration of 70-130 mg/dL, and a maximal postprandial capillary glucose concentration of 180 mg/dL [39, 41]. For approximating chronic levels of blood glucose over a 90-day period, the HbA1C test was developed to measure glycated proteins, and maintenance of HbA1c levels below 7% is considered optimal in most adults [42, 43]. The standard of care for DM includes regular blood glucose monitoring and HbA1c testing at least quarterly [39, 44, 45].

Treatment of T1DM primarily consists of rigorous blood glucose management by controlling dietary carbohydrate intake and supplementing β cell insulin secretion with exogenous insulin [39, 46]. Automated electronic insulin pumps are available, with the most recent generation of insulin pumps and glucometers able to communicate wirelessly thereby simplifying T1DM management [47]. It is anticipated that in the near future a “closed-loop” combination glucometer and insulin pump will be available [48-50], but until such a device is available the current method of dietary control, regular measurement of blood glucose, and injection of exogenous insulin remains the preferred method to manage T1DM [51].

Similar to persons with T1DM, persons with T2DM must monitor carbohydrate intake and regularly measure blood glucose. Although the first line treatment for T2DM is altering diet and increasing exercise, due to compliance issues diet and exercise interventions alone are rarely successful [52-54], and so pharmaceuticals to enhance insulin sensitivity or increase insulin secretion are also usually prescribed [46, 55]. The majority of people with T2DM do not initially require exogenous insulin supplementation

[56], but with time T2DM can worsen such that some persons develop a T1DM-like dependence on exogenous insulin [53, 57, 58].

I.B. Pancreatic Islets and the β Cell

The multiple types of cells responsible for the endocrine function of the pancreas are located in micro-organs embedded throughout the head, tail and body of the pancreas [59]. The “Islets of Langerhans” were first described as resembling islands by the medical student Paul Langerhans in 1869 [60, 61]. Endocrine islets are found in the pancreata of vertebrate animals, but insulin has been found in most animal species whether they have a pancreas or not [62, 63]. Pancreatic islets are surrounded by a membrane defining a boundary from the surrounding non-endocrine pancreatic cells, and islets are well supplied with blood via extensive capillaries passing within and throughout the islet [64, 65]. This high degree of vascularity allows the endocrine cells in the islet to actively sense signals from, and secrete signals into, the blood [66].

The β cell is one of five types of endocrine cells found in the islets of Langerhans. The other islet cell types – α, δ, PP and ε cells – secrete glucagon, somatostatin, pancreatic polypeptide and ghrelin, respectively [67, 68], but the β cell is the only physiologically relevant source of insulin in higher vertebrates [2]. Islet structure differs between organisms, for example the non-β cells of the islet surround the periphery of a mouse islet, while islet non-β cells are randomly distributed in the islet of a human [69]. Pancreatic β cells make up the majority of cells within pancreatic islets, with β cells comprising approximately 70-80% of the islet in humans [70].

I.B.1. β Cell Development and Proliferation

Although this work does not relate directly to β cell development or proliferation, an understanding of islet development is relevant to a full understanding of the signaling

pathways involved. The embryonic pancreas initially differentiates from the primitive intestinal endoderm. The pancreas develops into three distinct cellular sub-types: acinar cells, ductal cells, and endocrine cells [71, 72]. Differentiated acinar cells and ductal cells have a negligible effect on DM, but the pancreatic islets are indispensable to physiological management of metabolism [65]. When the pancreas is fully formed the endocrine islets form less than 3% of the total mass of the pancreas [65, 73, 74]. All five islet cell types differentiate from a common islet precursor cell, but the β cells express transcription factors that specifically regulate glucose sensing and insulin production and secretion. The maintenance of the expression of these β cell idiomatic proteins as well as the suppression of non-β cell genes is necessary for maintenance of euglycemia [75], as dedifferentiation of β cells and a corresponding decrease in insulin secretion has recently been suggested to contribute to the etiology of T2DM [76, 77].

Animal models do not perfectly replicate the behaviors and characteristics of human β cells. For example β cells continue to proliferate throughout the lifetime of a rodent, but human β cells do not [78]. The result is that in rodents pancreatic β cell population increases with age and generally corresponds to the overall size of the body for non-diabetic animals [79, 80]. Interestingly, in mouse models an increase in the rate of β cell proliferation is the first response to hyperglycemia, however when proliferation becomes impaired frank DM is the result [81]. Restoration of β cell proliferation ameliorates hyperglycemia and reverses DM in mice, however [82, 83]. The β cell populations in the pancreata of rodents also increase during pregnancy, however this increased proliferation ceases with parturition after which the additional β cells involute [84].

There are a number of key differences between humans and rodents with respect to β cell proliferation. In humans the β cells proliferate most rapidly in the gestational period just after initial pancreatic development, but β cell proliferation decreases to less than 5% of embryonic levels by six months of age [85]. In humans β cell proliferation

generally ceases during the third decade of life, but pancreatic β cell replication has been observed in adult human pancreata under certain circumstances [86-88]. For example, in an attempt to maintain normal physiological concentrations of blood glucose under insulin resistant conditions, the β cell's response to hyperglycemia is to increase insulin secretion via an increase in both size and number [82, 89-93]. In a recent study of cadaveric pancreata in both lean and obese persons it was found that obese persons had approximately 50% greater β cell mass compared to lean persons, and that this increase was due to a greater number of β cells [94]. Another notable exception to the relative lack of β cell proliferation in adult humans is pregnancy, since β cells in pregnant human females also proliferate [95]. As observed with rodents, the hormonal milieu of pregnancy stimulates proliferation of β cells in the pancreas of the pregnant female, but the nature of the proliferation differs. In human females the increase in β cell mass is due to an increased number of new, smaller islets, and unlike rodents evidence of post-partum islet remodeling via β cell apoptosis in post-partum human females has not been observed [96, 97].

Although the intracellular mechanism for β cell expansion has not been fully explained [98], certain hormones are known to play a role in β cell compensatory proliferation. Pancreatic β cells express receptors for growth hormone (GH) and insulin-like growth factor 1 (IGF-1), and the effect of hyperglycemia on β cell GH receptor knockouts shows that a lack of GH signaling in the β cell under hyperglycemic conditions results a lack of compensatory β cell hyperplasia [99]. An example of cell-cell signaling contributing to β cell proliferation is epidermal growth factor receptor (EGFR) signaling, which is required to form β cells *in utero* and it is also required for β cell compensatory proliferation with obesity or pregnancy [90].

In addition to proliferation, β cells also respond to hyperglycemia by increasing in diameter. Pancreatic β cell hypertrophy is a pro-survival response, and it is accompanied

by pro-survival gene upregulation [100]. As with proliferation there is hormonal signaling leading to β cell hypertrophy, specifically GLP-1 signaling [82]. Despite increased β cell function with hypertrophy, β cell survival is relatively impaired with larger size [91]. This may reflect the activation of pathways that favor survival over function, or it may be due to decreased resiliency of the individual β cell related to cell size [101], but β cell hypertrophy is primarily only suitable as a temporizing adaptation [91].

Unfortunately in DM-susceptible individuals pancreatic β cell proliferation and hypertrophy are insufficient to prevent disease progression [102]. So long as the β cells are capable of secreting sufficient insulin to dispose of excess blood glucose the individual will not progress to frank diabetes, but if β cell compensation should fail then the individual will progress from impaired glucose tolerance and hyperinsulinemia to uncontrolled hyperglycemia and DM [91].

I.B.2. β Cell Function and Insulin Secretion

Essential to the function of the pancreatic β cell is the ability to sense and respond to elevated blood glucose levels [103]. All cells in the body are able to take up glucose from the blood, however specialized glucose transporter proteins GLUT1 and GLUT2 ensure that the β cell is more sensitive to basal blood glucose levels than other cell types [104, 105]. Once glucose enters the pancreatic β cell it is phosphorylated by the enzyme glucokinase (GCK), both to begin the process of glycolysis and also to prevent the glucose from leaving the β cell [106]. Glycolysis begins in the cytoplasm but the mitochondria synthesize the majority of the energetic intermediate adenosine triphosphate (ATP) via enzymatic oxidation of molecules derived from the original glucose molecule [107]. The resultant increase in cellular ATP concentration triggers calcium flow into the cytoplasm from the extracellular space via ATP-linked potassium (KATP) ion channels [108], after which the calcium influx stimulates exocytosis of

perimembrane insulin granules and the first phase insulin response [103]. Blood glucose sensing and subsequent calcium signaling stimulate increased insulin synthesis, insulin processing, and insulin secretion, which leads to the second sustained phase of insulin response [109]. Glucose and calcium signaling in the β cell are inseparably connected to the ability of the β cell to maintain euglycemia.

Glucose is not the only trigger for β cell insulin secretion. Since depolarization of the β cell relies on KATP ion channels, extracellular increases in potassium concentration can cause depolarization and insulin release [110]. Potassium-stimulated insulin release (KSIS) is not suitable as a DM treatment modality [111], and thus KSIS is better understood as a tool for *in vitro* investigations of β cell function. Free fatty acids (FFAs) are another fuel source taken up from the blood by the β cell, and β cells secrete insulin in response to elevated serum FFAs [112]. FFAs are not an optimal fuel source for the β cell however because chronic lipid catabolism depletes the β cell of insulin [113]. Other simple sugars also stimulate insulin secretion, as fructose causes insulin secretion from the β cell albeit with a time delay compared to glucose [114, 115]. Pharmaceuticals such as the sulfonylurea class of drugs are able to directly stimulate insulin secretion via activation of the eponymous sulfonylurea receptor, and although sulfonylurea insulin secretagogue drugs are useful for treatment of T2DM their use is now deprecated due to concerns about β cell exhaustion [116, 117].

Pancreatic β cells are not responsive to metabolic signals only; they are also capable of increasing insulin synthesis and secretion depending on endocrine, neuronal, and other stimuli [118, 119]. Growth and lactation hormones secreted into the blood of pregnant females cause β cell proliferation and an increase in insulin secretion [119], but other hormones also have insulin effects. The intestinal hormone glucagon-like peptide 1 (GLP-1) directly stimulates the β cell to release insulin in response to a meal [120], and treatments that either extend the half-life of GLP-1 or provide a long-lasting GLP-1

homologue aid in glucose management for patients with T2DM [121]. Protein digestion promotes secretion of the stomach hormone gastrin which increases glucose stimulated insulin secretion, thereby enabling protein digestion to potentiate normal β cell response to post-prandial blood glucose elevations [122, 123]. Paracrine signaling within the islet also regulates β cell function; in humans paracrine release of acetylcholine by α cells primes the β cell for response to changes in blood glucose levels. In rodents α cells are not evenly distributed throughout the islet and therefore acetylcholine signaling to β cells is via a neuronal rather than paracrine mechanism [124]. Islet parasympathetic neurons are not the only relevant neurons to DM; the transient receptor potential vanilloid subfamily member 1 positive (TRPV1+) sensory neurons of the islet play a role in DM. TRPV1+ neurons are directly implicated in insulitis and autoimmune attack of β cells in T1DM, and for that reason islet TRPV1+ sensory neurons are being investigated as pharmacological targets for the prevention of T1DM [125, 126]. Beyond just glucose sensing, it is the interplay of metabolic, endocrine and neuronal factors that regulate β cell function and insulin secretion in response to nutrients and environment.

I.C. The Endoplasmic Reticulum in the β Cell

An individual β cell can synthesize one million insulin molecules per minute [127], and the accordant translational burden requires a well-developed endoplasmic reticulum (ER). The ER is the location of peptide synthesis and protein processing [128, 129], therefore the health of the ER is directly relevant to β cell function [130]. Significant disruption of β cell ER processes may activate intracellular stress signaling, leading to β cell apoptosis, diminished functional β cell mass, and eventually DM [131-133].

I.C.1. Endoplasmic Reticulum Stress

The specialized milieu of the ER lumen has specific requirements, and if these requirements are not met the result is ER stress [134]. ER stress in the pancreatic β cell has been established as a contributing factor to onset of both T1DM and T2DM [135, 136]. The ER lumen contains specialized protein chaperones to guide the folding of nascent peptides as they are synthesized [137, 138]. Chaperones require that conditions in the ER meet certain pH, temperature and ionic requirements [139-142], and one such requirement is a Ca^{2+} concentration orders of magnitude higher than the cytoplasm [143]. as a consequence of impaired chaperone activity, misfolded proteins aggregate and cause stress within the ER [144].

In addition to insufficient Ca^{2+} concentration, potential causes of ER stress include inflammation, long chain saturated fatty acid surfeit, extreme levels of reactive oxygen species, drugs that inhibit ER function such as tunicamycin and thapsigargin, and mutations causing an accumulation of misfolded proteins [128, 145-147]. The result of ER stress is that unfolded and misfolded proteins cannot be trafficked to the Golgi apparatus for intracellular delivery or extracellular secretion, so these protein aggregates must be cleared from the ER by other means [134]. This ER stress related blockage in protein and peptide processing results in the activation of the intracellular unfolded protein response (UPR) [144, 148], leading to either cell recovery or death (**Figure 2**).

I.C.2. Calcium in the β cell

A key determinant of the β cell's ability to fulfill its physiological role is strict regulation of ionic calcium [149-151]. When chronically overstimulated, Ca^{2+} homeostasis in the β cell is disrupted, and the internal stores of Ca^{2+} in the β cell are also affected [93, 152-155]. When ER Ca^{2+} concentration decreases calcium-dependent ER chaperone proteins such as calreticulin and calnexin are unable to perform their

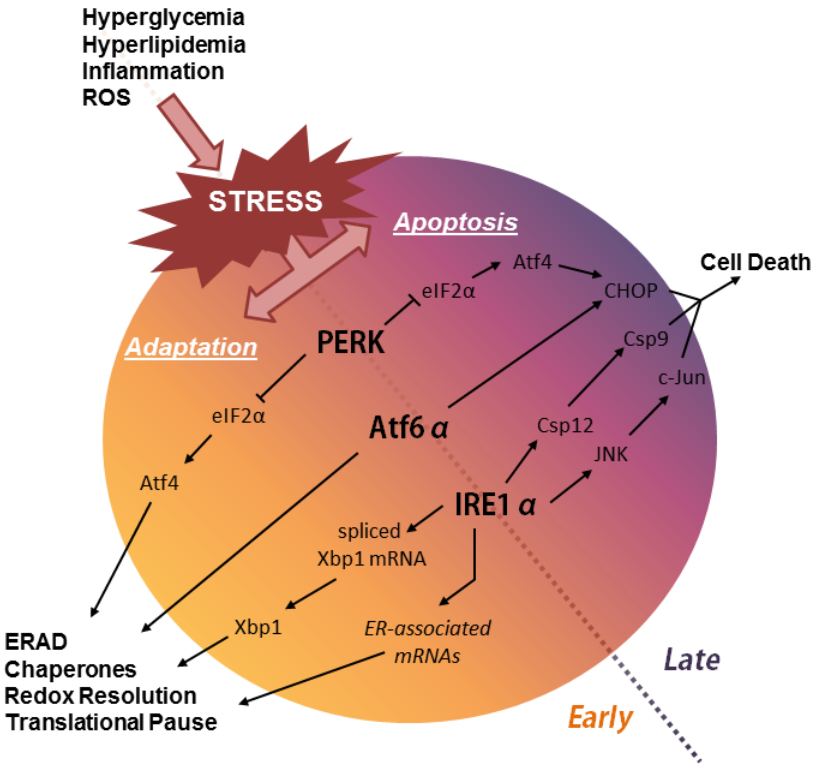


Figure 2. Endoplasmic Reticulum Stress and the Unfolded Protein Response.

When homeostasis of the ER is disrupted, unfolded proteins aggregate. The ER senses unfolded proteins and triggers ER stress response signaling pathways to respond to the accumulating stress. Early effects of ER stress response are adaptive, as the unfolded protein response (UPR) halts ordinary protein synthesis and increases the synthesis of ER stress resolution proteins. If the UPR is not able to resolve the stress, then apoptosis may be triggered via the internal apoptosis pathway [156].

function. Under normal conditions these chaperone proteins facilitate the production of a higher quantity and quality of synthesized proteins, but without sufficient Ca^{2+}, chaperone function is diminished [157]. Erosion of the steep Ca^{2+} concentration gradient between the ER lumen and cytosol therefore interferes with protein folding [136, 147, 158, 159], and the stress due to sustained ER Ca^{2+} dysregulation can trigger β cell death [146, 160-162].

Chronic hyperglycemia causes Ca^{2+} homeostasis disruption, dysregulation of intracellular signaling, derangement of peptide synthesis and impairment of β cell survival and function. Other factors that contribute to the erosion of the ER Ca^{2+} gradient are inflammatory cytokines, hyperlipidemia, and excessive generation of reactive oxygen species [163-165].

I.C.3. The Unfolded Protein Response

The unfolded protein response (UPR) is initially pro-survival as it attempts to resolve the cause of ER stress, but if the stress cannot be resolved the UPR can trigger apoptosis [128, 166]. When ER stress is initially detected, the cell responds by reducing the rate of transcription and translation to prevent the accumulation of additional toxic aggregates [134]. Not all peptides and protein synthesis is halted, however; the cell increases transcription and translation of proteins that aid in protein folding, protein trafficking, ER associated peptide degradation (ERAD), with the purpose of clearing the ER of harmful synthesis products [167]. If these attempts at recovery from ER stress fail, cells self-terminate by activating the C/EBP Homology Protein (CHOP) apoptosis pathway [145, 166].

Genetic studies in mouse models have informed critical components of the UPR in the β cell [168-170]. . If the UPR is not successful in resolving β cell ER stress, the result is loss of functional β cell mass and DM. One model illustrating the importance of the

UPR to β cell survival is a pancreas-specific knockout of Protein Kinase RNA-like Endoplasmic Reticulum Kinase (PERK). PERK maintains protein synthesis at a controlled rate, and pancreas-specific PERK knockout mice are prone to β cell ER stress, loss of functional β cell mass and DM [168]. Another mouse model unable to resolve ER stress also experience reduced functional β cell mass and DM, including CCAT-enhancer binding protein (C/EBP) transgenic mice, which also lose functional β cell mass due to irremediable ER stress [171]. Conversely, prevention of apoptosis due to ER stress via overexpression of the Apoptosis Repressor with Caspase Recruitment Domain (ARC) in the β cell maintains functional β cell mass and prevents DM [169]. A similar β cell survival advantage was also seen in the ER stress-resistant thioredoxin interacting protein (TXNIP) knockout mice [172]. Further studies of similar β cell ER stress pathways have also provided evidence that unresolved ER stress causes β cell apoptosis, and that relieving β cell ER stress can prevent apoptosis and DM [131-133, 139, 173].

I.D. Sarco-endoplasmic reticulum ATPase

Sarco-endoplasmic reticulum ATPases (SERCAs) are cellular calcium pumps responsible for maintaining the relatively high concentration of Ca^{2+} in the ER [174]. SERCA actively transports two calcium ions from the cytosol into the lumen of the ER per hydrolyzed ATP molecule (**Figure 3**) [175]. SERCA proteins are P-type ATPases with phosphorylation, calcium binding and nucleotide binding domains protruding from the cytosolic face of the ER membrane, and ten to eleven transmembrane loops creating a channel between the cytosol and the lumen of the ER [174, 176, 177]. SERCA is autophosphorylated at Aspartate-351 on the cytosolic face during the process of pumping calcium from the cytoplasm into the lumen of the ER, but this phosphorylation is understood to have only conformational effects [174, 176-178]. SERCA

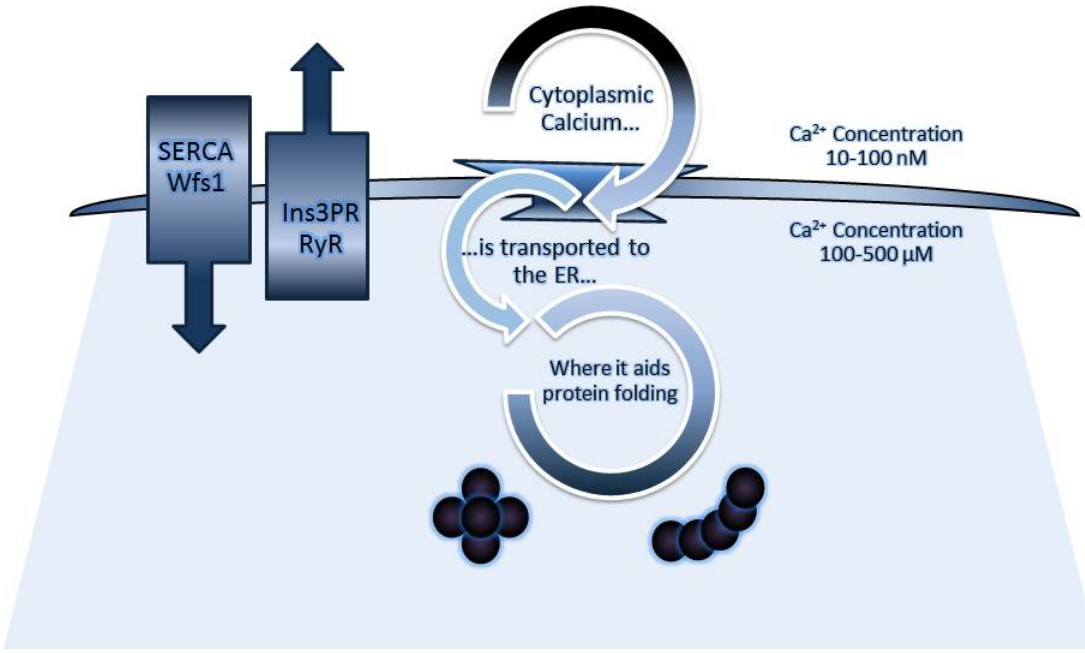


Figure 3. Endoplasmic Reticulum Calcium Transporters. Transport proteins move Ca^{2+} ions across the ER membrane both into and out of the ER lumen. The inositol triphosphate receptor (Ins3PR) and the ryanodine receptor (RyR) allow Ca^{2+} to move down its concentration gradient from the ER lumen to the cytosol. The SERCA family of ion pumps hydrolyze one ATP molecule to move two Ca^{2+} ions into the lumen of the ER. The precise function of wolframin (Wfs1) is not known, but it is known to be an ER membrane protein required for Ca^{2+} reuptake from the cytosol to the lumen of the ER.

overexpression under basal conditions may cause dysfunction and cell death in cardiac myocytes [179-181], but if the overexpression is in the context of previously diminished SERCA or in states of stress then the effect is pro-survival [182, 183].

There are multiple SERCA isoforms expressed in different tissues, with three isoforms expressed in the β cell [155]. Although the isoforms SERCA2a and SERCA3 are also present, the most prevalent isoform is SERCA2b [155, 184]. SERCA3 has been observed to decrease under diabetic conditions, but loss of SERCA3 is not sufficient to cause DM in SERCA3 knockout animals [185, 186].

I.D.1. Sarco-endoplasmic reticulum ATPase Isoform 2 (SERCA2)

SERCA2 isoforms are expressed throughout the body but SERCA2 has been most studied in cardiac tissue, and a mouse model overexpressing SERCA2 demonstrated increased cardiac function in both normal and diabetic animals [187]. A mouse model with deleted SERCA2 in all tissues is embryonic lethal, however a whole-body SERCA2 heterozygote mouse is viable [188]. These SERCA2 heterozygote mice demonstrate cardiac impairment, but the metabolic and potentially diabetic phenotype has not been extensively studied [189, 190]. Haploinsufficiency of SERCA2 in humans causes Darier's disease, which manifests in skin rashes and lesions [191], but there has been little study of susceptibility to DM in Darier's disease [192].

There are two SERCA2 isoform subtypes: SERCA2a primarily found in cardiac and muscle tissue, and SERCA2b which is expressed ubiquitously [193, 194]. The relevance of SERCA2a in the β cell has not been widely studied, but its comparatively low expression suggests that its function is less significant than the other two SERCA isoforms [155]. SERCA2b is classified as a "slow-twitch" SERCA isoform in comparison to the "fast-twitch" SERCA1a and SERCA2a isoforms [195]. SERCA2b has greater specificity for calcium transport than other SERCA isoforms, but it is also less

processive, which makes it ideal for maintaining a steep ER calcium gradient under normal cellular conditions [155, 185, 196].

SERCA2b is also unique among SERCA isoforms in that it possesses an eleventh transmembrane loop and a regulatory C-terminus tail within the lumen of the ER [197], allowing its activity to be modulated by intraluminal ER signaling [198]. The eleventh transmembrane domain is responsible for the increased affinity of SERCA2b for Ca^{2+} compared to other SERCA isoforms [199], whereas the intraluminal C-terminus allows isoform-specific regulation by factors within the lumen of the ER [177, 194, 200]. Decreased SERCA2b expression in the β cell is correlated with alterations in intracellular Ca^{2+} homeostasis, impaired insulin secretion, and cell death [201-203]. In fact direct interference with SERCA2 in the β cell can be fatal; the SERCA inhibitor thapsigargin is lethally toxic to β cells at millimolar concentrations, while SERCA2 interfering RNA causes irremediable ER stress due to calcium dysregulation [128, 147, 186, 203].

I.D.2. SERCA2 Regulation

The regulation of SERCA2 is complex, incorporating regulation of transcription, rate of translation and post-translational modifications [174, 204], as well as interactions with other proteins and signaling pathways [179, 198, 205, 206]. ER stress initially leads to an increase in transcription of SERCA2 isoforms in cardiac and neuroendocrine cell types, however sustained ER stress or cytokine signaling results in SERCA2 degradation [207-213]. This decrease does not eliminate SERCA2 expression entirely, but the concomitant ER Ca^{2+} dysregulation increases β cell vulnerability to apoptosis with further negative stimuli [155, 203].

I.D.2.i. SERCA2 Transcriptional Regulation

Knockdown of SERCA2 via interfering RNA causes translational upregulation of other Ca^{2+} pathways in response, which suggests an interconnection of Ca^{2+} sensing pathways and SERCA2 transcriptional regulation [214]. When vascular smooth muscle cells were treated *in vitro* with the irreversible SERCA inhibitor thapsigargin transcription and stability of multiple SERCA isoforms' mRNA increased, but this increase in SERCA message was abrogated with Ca^{2+} chelation, suggesting once again that Ca^{2+} signaling is required to upregulate *SERCA2* transcription in response to Ca^{2+} homeostasis disruption. The mechanism of this increase in *SERCA2* transcription does not require: active synthesis of new proteins, calcineurin binding, protein kinase C (PKC) activity, Ca^{2+}/calmodulin-dependent protein kinase (CAMK) activity or tyrosine kinase activity [215].

Peroxisome proliferator activating receptor (PPAR) proteins also upregulate the transcription of SERCA isoforms in response to metabolic signaling, but the pathways and degree of enhancement varies by tissue. The pharmaceutical compound etoxomir increases *SERCA2* transcription in cardiomyocytes, possibly via a mechanism involving the alpha isoform of PPAR (PPARα) [216], but in the β cell PPARα is not highly expressed [217]. PPAR isoform gamma (PPARγ) has also been shown to be a *SERCA2* transcriptional enhancer, and it is present in various cell types including the β cell [155, 218, 219].

Hormones and growth factors can also signal increase in *SERCA2* transcription however the mechanism by which each signal does so is isoform and tissue-specific. For example, thyroid hormone can signal skeletal- and cardio- myocytes to upregulate *SERCA2* transcription [220, 221], while platelet-derived growth factor (PDGF) causes increased *SERCA2* transcription in cardiomyocytes as well [222]. Estrogen and progesterone also enhance SERCA2 expression in cardiomyocytes and Sertoli cells, as

it has been observed that the lack of female hormones decreases *SERCA2* transcription and protein levels [223-226]. Finally, and most importantly in the study of DM, insulin signaling via the insulin receptor affects SERCA2b expression and *SERCA3* transcription in the pancreatic β cell itself [227, 228].

I.D.2.ii. SERCA2 Post-translational Regulation

In cardiomyocytes and in pancreatic β cells, CaMK isoform II (CaMKII) phosphorylation of Serine-38 of SERCA2 increases its activity, thereby assisting in maintenance of the ER Ca^{2+} gradient [229, 230]. Unfortunately in situations of chronic Ca^{2+} stimulation, it has been observed in myocytes that the loss of phosphorylation by CAMKII also decreases SERCA2 activity [230-232].

In cardiomyocytes SERCA2 activity may also be inhibited by modifications due to reactive nitrogen species [233, 234]. SERCA2 is the most resistant SERCA isoform to peroxidization, and at low concentrations reactive oxygen species enhance SERCA2 function [235, 236]. This enhancement is not unlimited, as the nitration of SERCA2 residues Tyrosine-294 and 295 is correlated with decreased SERCA2 levels in skeletal muscle [174, 237-239]. When sulfonylation of Cysteine-674 accompanies these inhibitory nitrations, SERCA2 is further inhibited [240].

In addition to nitration and sulfonylation, in the presence of NO or other oxygen radicals Cysteines-498, 524, 613 and 674 become glutathionylated [174]. These glutathionylation modifications have been observed in vascular smooth muscle cells, and the modifications temporarily increase SERCA function in response to NO signaling. Under conditions of chronic stimulation, however, oxidation, of Cysteine-674 prevents this upregulation via glutathionylation [241, 242]. When PC12 pheochromocytoma cells are treated *in vitro* with EGTA to remove Ca^{2+}, treated with DTT to inhibit disulfide bridge formation, or treated with tunicamycin to prevent glutathionylation, post-translational

modification by reactive oxygen and nitrogen species in a chronically oxidative environment impairs SERCA and calcium homeostasis [209]. In this way concentrations of reactive oxygen species that might be considered normal facilitate SERCA2 function, whereas higher concentrations of reactive oxygen species as seen with inflammation or chronic stimulation impairs SERCA2 function [243].

I.D.2.iii. SERCA2 Protein-Protein Interactions

In addition to the signaling pathways that regulate SERCA2 via post-translational modifications, ER membrane and ER lumen proteins are able to influence the activity of SERCA2 via physical interactions. This intraluminal regulation is thought to occur especially in the presence of ER reactive oxygen species [244]. SERCA does not act independently; interactions with other proteins assist in the coordination of ER Ca^{2+} regulation.

Proteins known to regulate SERCA2a activity in cardiomyocytes are sarcolipin and phospholamban, but they are not found in the pancreatic β cell [222]. The anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) is embedded in the ER membrane and cooperates with SERCA2 to prevent apoptosis in the cardiomyocyte with Ca^{2+} dysregulation [174, 222]. Calcium sensing proteins also affect SERCA2 function; the ER intraluminal protein calreticulin contributes to the adaptive glycosylation of the 11^{th} transmembrane loop of SERCA2b [205], which modulates SERCA2b activity during Ca^{2+} oscillations. Like calreticulin, the chaperone proteins calnexin and ER protein 57 (GRP-58) also interact with luminal domains of SERCA2 to dampen extreme fluctuations in ER Ca^{2+} [198, 206].

I.D.2.iv. Translational Regulation of SERCA2

Little is known about the translation of SERCA2 from mRNA, and if there is translational regulation of SERCA2 it may vary by isoform or tissue type. When neuronal

cells were treated with thapsigargin and allowed to recover, there was a doubling of SERCA2b mRNA but levels of SERCA2b protein were unchanged [245]. In contrast, SERCA2a protein levels in skeletal muscle and in cardiomyocytes roughly equal SERCA2a mRNA levels [246]. Since the SERCA2b isoform dominates in the β cell, if one were to draw a conclusion from these limited studies it would be that an increase in SERCA2b mRNA does not necessarily result in an increase in SERCA2b protein levels, and that both protein and mRNA levels should be verified independently when possible.

I.D.3. Summary of SERCA2

Of the proteins in the SERCA family of Ca^{2+} pumps, the SERCA2 types are the most widely expressed throughout the body [187]. SERCA2b is the most highly expressed isoform in the β cell, followed by SERCA3 and SERCA2a, but it is SERCA2b that is required for normal ER Ca^{2+} homeostasis [155]. Without the steep ER Ca^{2+} gradient maintained by SERCA2b β cell function becomes impaired [186], and if the loss of functional β cell mass is sufficient the result is DM in the organism as a whole.

I.E. Pancreatic and Duodenal Homeobox Protein 1

Pancreatic and duodenal homeobox protein 1 (Pdx-1), previously referred to in the literature as IPF-1, IUF-1, IDX-1, STF-1 and GSF, was discovered by multiple investigators at approximately the same time, though each group described separate functions for this transcription factor [247, 248]. In vertebrates Pdx-1 is required for embryonic development of the pancreas [249], and in combination with the homeobox protein NK6 Homeobox protein 1 (Nkx6.1) Pdx-1 is also the defining transcription factor of the β cell [250, 251]. Pdx-1 regulates several key aspects of β cell function, including sensing of blood glucose, mitochondrial function, ER health, insulin synthesis and insulin

secretion [252-258]. In addition to the β cell Pdx-1 is expressed in islet δ cells and in select regions of the hypothalamus [259].

I.E.1. Pdx-1 and Pancreatic Organogenesis

Pdx-1 expression begins *in utero* in the foregut of the mouse at day 8.5, and this expression results in the separation and differentiation of the embryonic pancreas. Pdx-1 is then expressed in all cells of the pancreas until embryonic day 9.5, when pan-pancreatic Pdx-1 expression ceases [248]. As of embryonic day 13 and until the end of life, Pdx-1 is thereafter expressed primarily in β cells [259].

Lack of functional Pdx-1 results in pancreatic agenesis in mice and it is lethal shortly after birth [260, 261]. Haploinsufficiency of Pdx-1 in humans causes Maturity Onset Diabetes of the Young 4 (MODY4), but this condition is extremely rare [262, 263]. Homozygous deletion of Pdx-1 results in human pancreatic agenesis, and only two confirmed cases have been observed [260, 261, 264, 265].

I.E.2. Pdx-1 and β Cell Identity

Pdx-1 is essential to both pancreatic development and β cell maturation [71, 262]. Along with other transcription factors Pdx-1 regulates v-Maf avian musculoaponeurotic fibroscarcoma oncogene homolog A (MafA), which contributes to the unique epigenetic signature of the β cell [266, 267]. Pdx-1 is also critical to mature β cells where it enhances transcription of proteins essential to β cell function and proliferation [248]. The transcriptional targets of Pdx-1 include glucose transporter 2 (GLUT2) [258], glucokinase [252], insulin genes 1 and 2 (*INS1*, *INS2*) [253, 254], homeobox factor Nkx6.1 [268], nuclear-encoded mitochondrial factor A (TFAM) [257], as well as other proteins important to glucose sensing, insulin synthesis, insulin secretion and β cell survival [269]. Pdx-1 expression is also required for β cell proliferation [248, 270], and without

Pdx-1 compensatory glucose-stimulated β cell hyperplasia is impaired [271]. Equally important to its other roles, Pdx-1 also regulates the expression of ER stress related proteins activating transcription factor 4 (Atf4) [255], and Wfs1 [256].

I.E.3. Pdx-1 Regulation

The regulation of Pdx-1 is complex with many different factors affecting its transcription, activity or degradation [248]. Pdx-1 activity tends to be increased by glucose signaling, insulin stimulation, and short-term cellular stress, whereas chronic β cell stress leads to decreased Pdx-1 levels [248, 272-274].

I.E.3.i. Regulation of Pdx-1 Transcription

The Pdx-1 protein is encoded by the *Ipf1* gene, and the *Ipf1* promoter has binding sites for multiple types of DNA binding motifs, thus protein complexes rather than individual transcription factors are required to enhance Pdx-1 transcription [248]. During development and β cell differentiation the complexes of transcription factors that enhance Pdx-1 transcription include: hepatic nucleocyte factors 3 beta and 1 alpha (HNF-3β, HNF-1α) [275, 276], NeuroD (also called β2) [277], upstream stimulatory factor (USF) [278], PPARγ [251], and transcription factor specificity protein 1 (SP1) [279]. HNF-3β is of particular interest because it is itself transcriptionally upregulated by Pdx-1, thus forming a positive feedback loop, as levels of both transcription factors are positively correlated [280]. The propagation of extracellular signaling to the nucleus results in either enhanced transcription of Pdx-1 to increase protein levels or prevention of Pdx-1 degradation [281, 282].

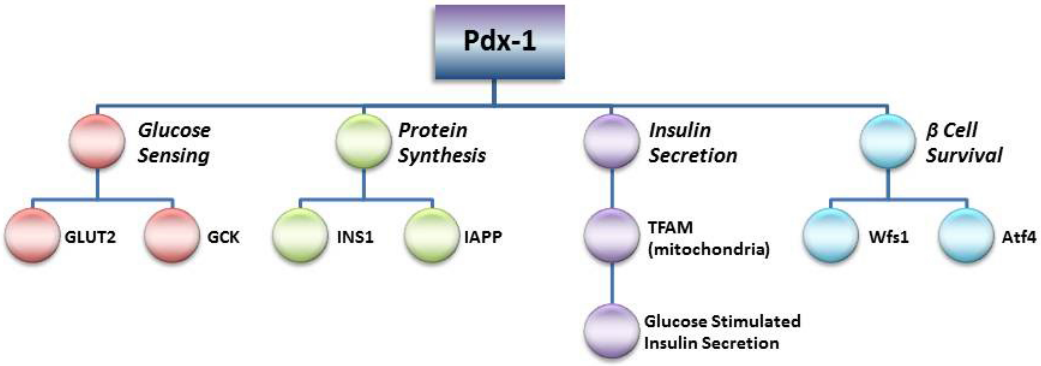


Figure 4. Pdx-1 transcription target proteins. Pdx-1 enhances transcription of proteins involved with every facet of β cell function. Targets that affect glucose sensing include glucose transporter 2 (GLUT2) and glucokinase (GCK). Pdx-1 increases the synthesis of proteins intended for secretion outside of the cell including insulin (INS1) and amylin (IAPP), while Pdx-1 also enhances the mitochondrial transcription factor A (TFAM) which helps the mitochondria trigger secretion via ATP synthesis. In addition, Pdx-1 enhances transcription of proteins that prevent maladaptive ER stress including Wfs1 and Atf4.

I.E.3.ii. Regulation of Pdx-1 Activity

Pdx-1 is post-transcriptionally modified in order to affect either its activity or its intracellular localization. Pdx-1 activity is upregulated by conditions that call for an increase in insulin synthesis, and factors that upregulate Pdx-1 activity include: p38 MAP kinase (p38) [281], phosphoinositide 3 kinase (PI3K) [272], stress-activated protein kinase 2 (SAPK2) [283], atypical protein kinase C zeta (PKCζ) [284], and follistatin [285]. These proteins cause an activating phosphorylation of Pdx-1, thereby enhancing its ability to bind to target gene promoters [248, 286].

It is not only intracellular signaling that increases the effect of Pdx-1 in the β cell; hormones and metabolic signaling also play a role. Basic Fibroblast Growth Factor (bFGF) [287], triiodothyronine (T3), heparin-binding EGF-like growth factor (HB-EGF) [274], insulin [288], TNFα and GLP-1 signaling increase Pdx-1 transcription [274]. Nutrients and vitamins that upregulate Pdx-1 transcription in the islet include biotin [289] and nicotinamide [287], while glucose increases Pdx-1 activity by causing its phosphorylation via the p38 mitogen-activated protein kinase pathway [248, 283]. The fact that many of the hormonal factors that enhance Pdx-1 transcription and therefore contribute to β cell proliferation are growth factors is logical, inasmuch as β cell mass has a positive correlation to body mass in general [80]. Contrariwise, glucocorticoids interfere with Pdx-1 transcription by negatively affecting HNF-3β and thereby reducing Pdx-1 transcription [248], so not all humoral signaling serves to increase Pdx-1 levels or activity in the β cell.

I.E.3.iii. Regulation of Pdx-1 Nuclear Localization

As a transcription factor, Pdx-1 must be in the nucleus to affect cellular function. Pdx-1 has a well-characterized nuclear location signal RRMKWWK [282], and the small GTPase protein Ran insures that Pdx-1 remains in the nucleus so long as the nuclear

localization signal is accessible [290]. Shuttling Pdx-1 out of the nucleus effectively silences it, and indeed when Pdx-1 is located in the cytosol it is degraded by the proteosome [291, 292]. Oxidative and inflammatory stress in the β cell causes activation of the c-Jun N-terminal kinase (JNK) pathway, which in turn leads to an inactivating phosphorylation of Pdx-1 and subsequent nuclear export [293]. The propagation of extracellular signaling to the nucleus results in either enhanced transcription of Pdx-1 or in the prevention of Pdx-1 degradation, both leading to an increase in Pdx-1 protein levels [281, 282].

I.E.4. Pdx-1 and β Cell Stress

Under oxidative stress, glycotoxic and inflammatory conditions of the diabetic milieu the expression of Pdx-1 is impaired and Pdx-1 levels decrease [294, 295]. When Pdx-1 levels decrease, β cell ER stress increases, which triggers the UPR [170]. Initially the UPR is adaptive, as the decrease in insulin synthesis burden putatively allows a stressed β cell to recover [156], but in the chronic condition the loss of Pdx-1 leads to loss of functional β cell mass, and under these circumstances the loss of Pdx-1 adds functional insult to cellular injury [136, 295].

By downregulating Pdx-1 the chronically stressed β cell forfeits the ability to proliferate [296]. Eventually failure to manage the β cell population occurs by cell death along with lack of proliferation, and the glycemic load worsens for β cells that still function, creating a vicious cycle of dysfunction and death [297, 298].

I.F. Peroxisome Proliferator-Activated Receptor γ

PPARγ is a type 2 ligand-dependent nuclear receptor present in the β cell, and it is the drug target of the thiazolidinedione (TZD) class of pharmaceuticals [299]. PPARγ is often complexed with the retinoid X receptor transcription factor (RXR) when active, and

although PPARγ is best known for its effects in adipocytes and hepatocytes [300, 301], the role of PPARγ in β cell function is highly significant [302, 303].

I.F.1. PPARγ Regulation

Although increasing the expression of PPARγ is one way of increasing its activity [304], PPARγ activity is also regulated by post-translational modification [305]. Phosphorylation of PPARγ at Serine-273 via cyclin-dependent kinase 5 (CDK5) does not prevent PPARγ from binding to the PPAR response element (PPRE), but it does prevent PPARγ enhancement of target gene transcription [306, 307]. The mechanism of this inhibition via phosphorylation at Serine-273 of PPARγ is unknown, but one possible explanation is that the added phosphate group prevents binding of PPARγ co-factors due to steric or electrostatic interference.

I.F.2. PPARγ Function in the β Cell

In order to promote transcription of target genes, PPARγ requires ligands to complex with it, particularly RXR [308, 309]. The structure of PPARγ includes a large binding pocket which could accommodate a variety of cofactors [310, 311], and proteins it is known to interact include those in lipid-related signaling pathways like C/EBP, sterol regulatory element binding protein 1, PPAR co-activator 1, and hepatic nuclear factor 4 [312]. Additional ligands found to complex with PPARγ include transcription factor complex-forming proteins such as steroid receptor co-activating factor 1, Creb-binding protein, E1A binding protein p300 , as well as fatty acids, prostanoid 15-deoxy$\Delta^{12,14}$PG J2, prostaglandins, leukotrienes and eicosanoid molecules [300, 310, 313, 314]. The binding of cofactors increases PPARγ activity and provides specificity to PPARγ transcriptional enhancement.

PPARγ is widely distributed in many tissue types throughout the body, but its role in the β cell is related to β cell function and identity [217, 251, 315]. PPARγ in the β cell also maintains euchromatin structure and plays a role in the mitigation of ER stress [303]. Under normal metabolic conditions PPARγ inhibits β cell proliferation in favor of maturation, however when fed high fat diet or after partial pancreatectomy PPARγ enhances proliferation by upregulating Pdx-1 and Nkx6.1 [251, 316].

In the β cell PPARγ increases the transcription and expression of Pdx-1, Nkx6.1, GCK and GLUT2 after partial pancreatectomy [251]. PPARγ also decreases the expression of cytokines and the inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), thereby promoting cell survival and function [146, 314, 317]. PPARγ contributes to ER Ca^{2+} homeostasis by increasing *SERCA2* transcription [201]. Levels of PPARγ are not decreased under conditions of diabetic stress in the β cell; indeed PPARγ levels have been seen to increase in the β cell when exposed to the diabetic milieu [93]. of particular interest in the present work is the binding of PPARγ to the *SERCA2* promoter [155].

With hyperglycemia, CDK5 phosphorylates PPARγ at Serine-273 and inhibits PPARγ-mediated transcriptional effects. Interestingly this post-translational modification does not impact binding of PPARγ to a gene promoter [318].

I.F.3. PPARγ Pharmaceuticals

The TZD drug pioglitazone maintains the activity of PPARγ both by inhibiting phosphorylation of Serine-273 by CDK5 as well as forming a direct complex with PPARγ [155, 300, 306, 319]. Pioglitazone is a second generation TZD most often prescribed in combination with other pharmaceuticals to aid in managing hyperglycemia with T2DM [320, 321]. The primary mechanism of action for the TZD class of drugs for glycemic control has until now been thought to be insulin sensitization of adipose tissues via its

dual agonism of PPARα and PPARγ [319], but data published by this laboratory as well as others have demonstrated that TZD drugs also have beneficial effects in the β cell itself [155, 322, 323].

Since their introduction different TZD drugs have come under scrutiny for various safety concerns. Troglitazone was the first TZD removed from the market for concerns over hepatotoxicity [300]. Second-generation TZD drugs did not show evidence of hepatotoxicity [324], however a new concern regarding possible cardiac side effects with rosiglitazone arose [325, 326]. Rosiglitazone remains available in the U.S. despite an increased risk of myocardial infarction [327], as does pioglitazone despite an association with bladder cancer [328], although both drug types carry FDA warning labels [329].

I.G. Forkhead Box Protein O1 in the β Cell

Forkhead box protein O1 (FOXO1) is a transcription factor that enhances expression of genes related to fuel production and storage [330]. FOXO1 is found in multiple tissue types however its effects in the hepatocyte, adipocyte and pancreatic β cell are most relevant to the study of DM. In the β cell FOXO1 is noted as a pro-survival and pro-differentiation factor but counterintuitively also as a particularly potent antagonist of Pdx-1 [331].

FOXO1 has long been considered the opposite of Pdx-1 in the β cell, and except that both proteins promote β cell survival the two are mutual antagonists [332]. Insulin signaling inhibits FOXO1 by the protein kinase B pathway, which maintains FOXO1 localization in the cytoplasm away from its target genes in the nucleus [333]. Oxidative and inflammatory stress in the β cell causes activation of the JNK pathway and FOXO1, leading to downstream inactivating phosphorylation of Pdx-1 and Pdx-1 nuclear export [293]. FOXO1 further inhibits the transcription of Pdx-1 by interfering with Hnf-3β and PPARγ enhancement of *IPF1* transcription [334, 335]. Reduction in Pdx-1 levels inhibits

β cell proliferation, so while FOXO1 improves individual β cell survival it impairs overall functional β cell mass [336].

Chapter II. Results

II.A. Pdx-1 and SERCA2b Levels Decrease Under Diabetic Stress

Pdx-1 levels decrease under hyperglycemic and inflammatory conditions, as in the diabetic milieu of the β cell [248]. SERCA2b levels also decrease in the β cell under diabetic conditions [155], so it was our goal to test whether alterations in Pdx-1 and SERCA2b expression in the β cell were correlated. Immunoblot and quantitative PCR were used to measure β cell Pdx-1 and SERCA2 expression in cell culture and in islets isolated from mouse models of DM and cadaveric human donors with and without T2DM.

II.A.1. Pdx-1 and SERCA2b Levels in the db/db Diabetic Mouse Model

Our previous work has shown significantly decreased expression of SERCA2b mRNA and SERCA2 protein in islets isolated from C57BLKs/J-db/db (db/db) mice [144, 155]. To investigate the potential correlation between Pdx-1 and SERCA2b expression in the β cell in diabetic animals, islets were isolated from 12-week-old db/db mice and heterozygote littermate controls. In a preliminary experiment, Pdx-1 and SERCA2b protein were decreased in db/db islets compared to controls (**Figure 5A**). Similar findings were observed measuring Pdx-1 and SERCA2b mRNA levels (**Figure 5B**).

II.A.2. Pdx-1 and SERCA2b Levels in Cadaveric Diabetic Human Islets

Next, mRNA was isolated from cadaveric islets from donors with Type 2 diabetics and donors without diabetes. Donors with a history of T2DM showed decreased average mRNA of both Pdx-1 and SERCA2b (**Figure 6A**). Levels of SERCA2b and Pdx-1 mRNA were graphed as a line with the X-coordinate corresponding to SERCA2b mRNA and the Y-coordinate corresponding to Pdx-1 mRNA. A significant linear relationship was

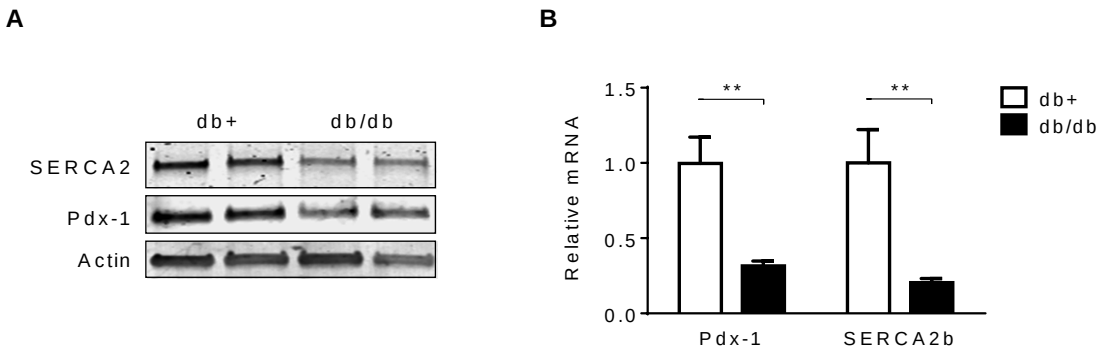


Figure 5. Pdx-1 and SERCA2b levels are decreased in db/db mice compared to db+ littermate controls. Protein and RNA were isolated from 12-week old db/db and db+ control mouse islets for measurement of relative Pdx-1 and SERCA2b expression. A) Protein immunoblot was performed using antibodies against SERCA2, Pdx-1, and actin loading control. B) Reverse-transcribed RNA was subjected to real-time PCR for quantification of SERCA2b and Pdx-1 transcript levels. Indicated comparisons are significantly different (**$p < 0.01$) by multiple t-test with Sidak-Bonferroni post-test correction. Panel A, islets from 3 db/db and 3 db+ mice were pooled, with 100 pooled islets per lane displayed as a representative immunoblot. Panel B, pooled islets from 3 db/db and 3 db+ mice were used for quantitative PCR, with 7 db+ and 9 db/db qPCR samples total. Results are displayed as the means ± S.E.M.

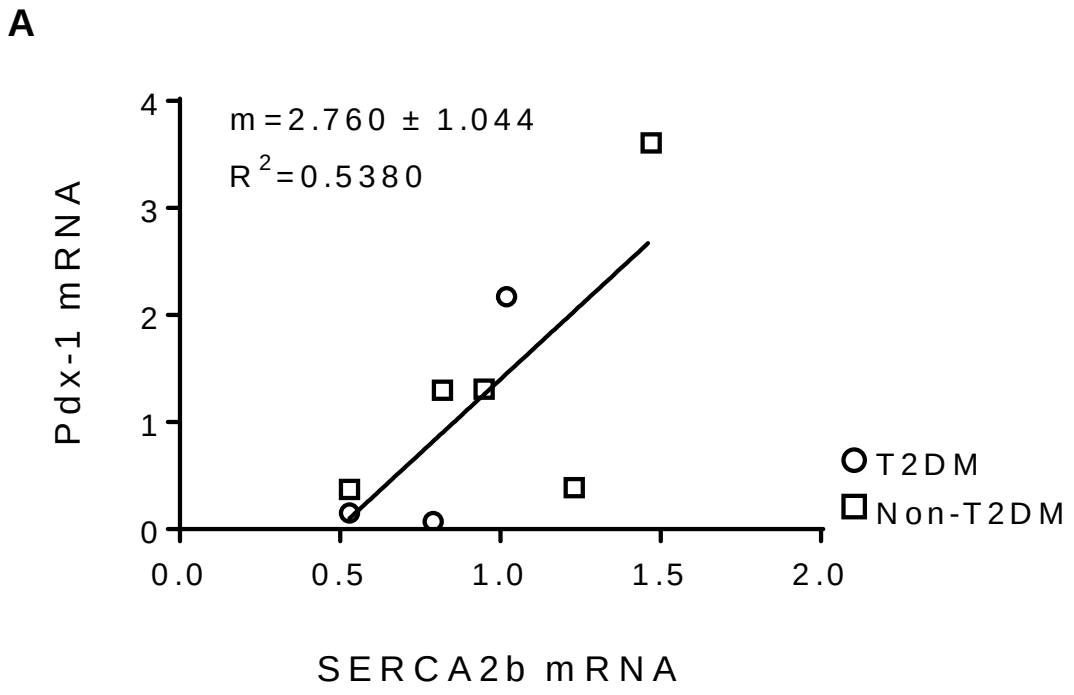


Figure 6. Pdx-1 and SERCA2b expression levels are correlated in cadaveric human donor islets. Human islets from diabetic and non-diabetic human donors were lysed and mRNA levels for Pdx-1 and SERCA2b were measured via quantitative PCR. A) Pdx-1 and SERCA2b mRNA levels were graphed as points on the Y-axis and X-axis with the Best-fit line calculated from the qPCR results. Islets isolated from 4 males, 4 females; 3 T2DM and 5 non-DM; average age 48 years and average BMI 26. Indicated positive correlation is significant ($p < 0.05$) as calculated by Best-fit analysis.

observed with a slope (m) of 2.760 ± 1.044 and a coefficient of determination (R^2) value of 0.5380, suggesting that mRNA expression levels of Pdx-1 and SERCA2b in human islets are positively correlated (**Figure 6A**).

II.A.3. Pdx-1 and SERCA2b Levels in an *In Vitro* Model of Diabetes Mellitus

Previous work has demonstrated a decrease in SERCA2b expression using an *in vitro* model to mimic the pro-inflammatory milieu of diabetes [155]. INS-1 832/13 rat insulinoma cells were treated with 25 mM glucose (high glucose, or HG) combined with 5 ng/ml of interleukin-1β (IL-1β) for 16 and 24 hours, after which protein and mRNA levels of Pdx-1 and SERCA2b were measured. At 16 hours post HG+IL-1β treatment, decreased Pdx-1 and SERCA2b protein levels were observed, with a representative blot and quantification shown. The results of 24 hours of HG+IL-1β were not significantly different from results observed at 16 hours (**Figure 7A-B**).

Differences in mRNA expression were also measured at 16 and 24 hours. At 16 hours HG+ IL-1β treatment Pdx-1 mRNA decreased to 50% expression, whereas SERCA2b mRNA did not significantly decrease, but after 24 hours of HG+IL-1b stress SERCA2b mRNA also decreased to approximately 50% of untreated levels (**Figure 7C**).

Because the insulin gene is a known transcriptional target of Pdx-1 [337], quantification of pre-insulin mRNA was measured to confirm a loss of transcriptional enhancement by Pdx-1 with HG+IL-1b stress. Decreased pre-insulin mRNA expression was observed in correlation with decreased Pdx-1 (**Figure 7D**). In contrast, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is not a Pdx-1 target [338], therefore GAPDH mRNA was measured to investigate whether non-Pdx-1 target proteins had a similar correlation to pre-insulin and SERCA2b. No significant change in GAPDH expression was observed with HG+IL-1β treatment (**Figure 7D**).

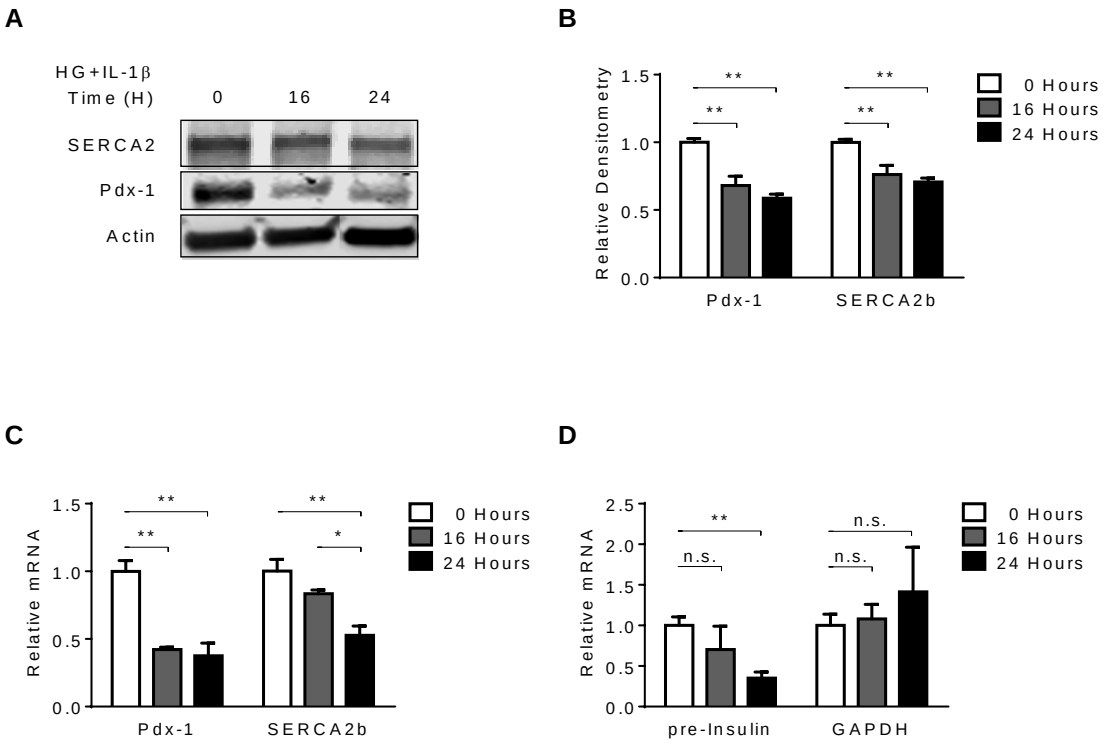


Figure 7. Levels of Pdx-1 and SERCA2b protein and mRNA are decreased in an *in vitro* model of the diabetic milieu. INS-1 832/13 rat insulinoma cells were treated with 25 mM glucose and 5 ng/ml IL-1β (HG+IL-1β) for 16 and 24 hours. A-B) Immunoblot was performed using antibodies against SERCA2, Pdx-1, and actin. Quantitative protein levels are shown graphically. C-D) Reverse-transcribed RNA was subjected to real-time PCR for quantification of SERCA2b, Pdx-1, pre-insulin, and GAPDH transcript levels. Indicated comparisons are significantly different (*$p < 0.05$, **$p < 0.01$) or not statistically significant ($^{n.s.}p > 0.05$), by ANOVA with multiple comparisons. n=4 or more in all panels. Results are displayed as the means ± S.E.M.

II.B. Knockdown of Pdx-1 Causes Intracellular Calcium Dysregulation

Our results demonstrate that decreased Pdx-1 and SERCA2b expression under diabetic conditions are correlated, but evidence of a direct relationship was lacking. In order to ascertain if the loss of Pdx-1 was causally related to diminished SERCA2b in the β cell, an *in vitro* strategy of Pdx-1 small interfering RNA was undertaken.

II.B.1. Knockdown of Pdx-1 Decreases SERCA2b Protein and mRNA

Adenoviral siRNA-mediated knockdown of Pdx-1 was performed in INS-1 832/13 cells, which express Pdx-1 at high levels under normal conditions [339, 340]. In the INS-1 cells treated with siRNA adenovirus, Pdx-1 levels were reduced by approximately 90%, resulting in a 50% decrease in SERCA2b protein (**Figure 8A-B**).

Levels of SERCA2b mRNA transcript after Pdx-1 knockdown were quantified, and with an approximately 90% Pdx-1 decrease in mRNA, there was an approximate decrease of 60% in SERCA2b mRNA. There was no significant decrease in SERCA2b mRNA expression with a random sequence siRNA adenovirus (**Figure 8C**). Taken together, these protein and mRNA results demonstrate that β cell SERCA2b levels are decreased with small interfering RNA knockdown of Pdx-1.

II.B.2. Knockdown of Pdx-1 Changes the Ratio of Cytosolic/ER Ca^{2+}

Loss of Pdx-1 alters β cell Ca^{2+} compartmentalization leads to decreased ER Ca^{2+} and increased cytosolic Ca^{2+} levels, consistent with a loss of mediated Ca^{2+} influx into the lumen of the ER. INS-1 cells were transduced with siPdx-1 adenovirus or control siRNA then incubated with Fura 2-AM Ca^{2+} dye to measure cytosolic Ca^{2+} levels. The ratio of 340/380 wavelength excitation is correlated with cytoplasmic Ca^{2+} concentration, and this ratio showed that Pdx-1 knockdown significantly increased Ca^{2+} levels within the cytosolic compartment (**Figure 9**).

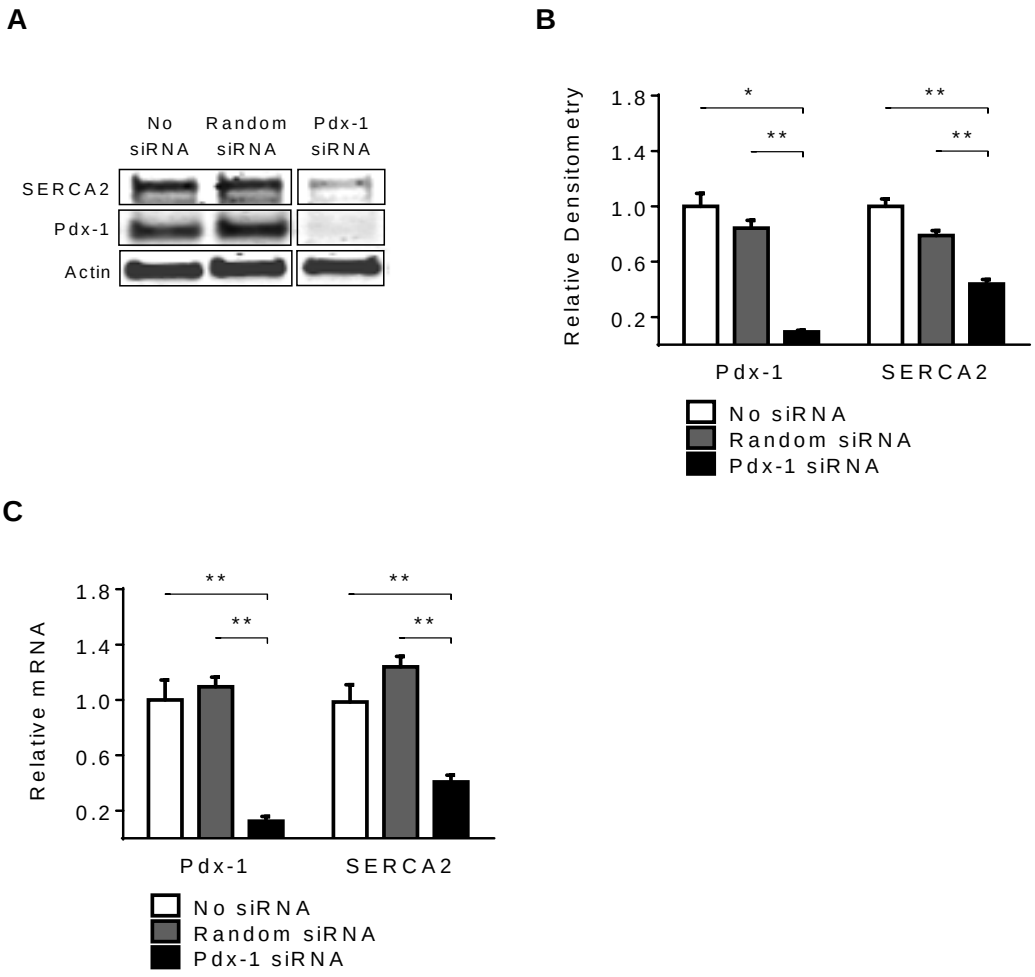


Figure 8. Pdx-1 siRNA in INS-1 cultured cells. INS-1 832/13 rat insulinoma cells were transduced with an adenovirus that expressed siRNA against Pdx-1. A-B) Immunoblot was performed using antibodies against SERCA2, Pdx-1, and actin. Quantitative protein levels are shown graphically. C) RNA was subjected to real-time PCR for quantification of SERCA2b and Pdx-1 transcript levels. Indicated comparisons are significantly different (*$p < 0.05$, **$p < 0.01$) by one-way ANOVA with multiple comparisons. n=4 or more. Results are displayed as the means ± S.E.M.

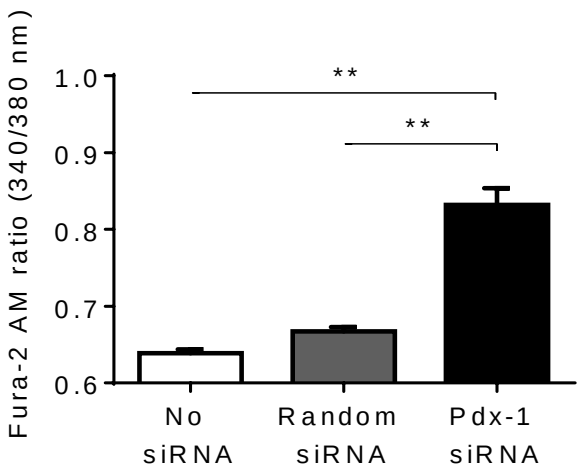


Figure 9. Cytoplasmic Ca^{2+} measurement with Pdx-1 siRNA using Fura-2AM fluorescent dye. The Fura 2-AM 340/380 fluorescence ratio was measured as described in the Experimental Procedures, in untreated INS-1 cells and in INS-1 cells transduced with siPdx-1 or random siRNA adenovirus. Indicated comparisons are significantly different (**$p < 0.01$) by ANOVA with multiple comparisons. n=at least 3 random fields, with at least 20 cells per field. Results are displayed as the means ± S.E.M.

II.B.3. Knockdown of Pdx-1 Decreases the Concentration of ER Ca^{2+}

To assess the impact of Pdx-1 loss on ER Ca^{2+} levels, INS-1 cells were transduced with an ER-targeted D4ER adenovirus in parallel with a siPdx-1 or random siRNA adenovirus. Fluorescence lifetime imaging microscopy (FLIM) was performed as outlined in the Experimental Procedures [341]. Using this strategy, an increase in the lifetime of the enhanced cyan-fluorescent protein (ECFP) donor indicates less FRET efficiency and therefore lower ER Ca^{2+}.

Random fields were imaged in each dish with each treatment condition. Representative micrographs of transduction with either random siRNA or siPdx-1 adenovirus provide visual representation of the functional consequence Pdx-1 knockdown (**Figure 10A**). When fluorescence lifetime was quantified, ECFP donor lifetime increased significantly from 1.72 ± 0.0213 ns with random siRNA to 1.83 ± 0.0214 ns with siPdx-1, consistent with reduced ER Ca^{2+} with Pdx-1 knockdown (**Figure 10B**).

II.C. Pdx-1 Enhances Transcription at the *SERCA2* Promoter

Pdx-1 is known to bind to TA-rich regions including TAAT, ATTA and TAAAT sequences in the promoters of target genes [342, 343]. The *SERCA2* promoter has multiple putative Pdx-1 binding sites, and so we investigated whether exogenous Pdx-1 supplementation increased activity at the *SERCA2* promoter.

II.C.1. Pdx-1 Overexpression in Fibroblasts Increases SERCA2b

NIH-3T3 mouse fibroblast cells express SERCA2b but do not natively express Pdx-1. To test the relationship between Pdx-1 and SERCA2b expression directly, NIH-3T3 fibroblasts were transduced with Pdx-1 adenovirus which resulted in an approximate

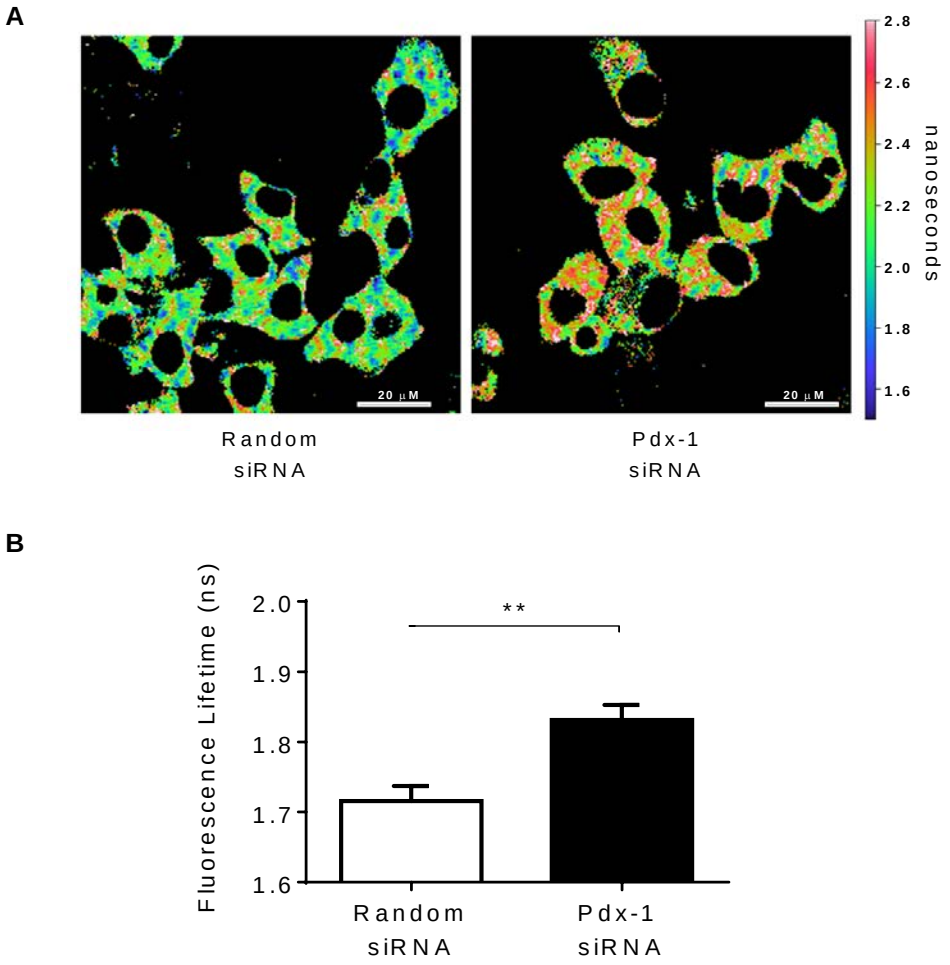


Figure 10. Direct measurement of ER Ca2+ using an ER-targeted D4 fluorescent protein. INS-1 cells were transduced with a D4ER Ca^{2+} reporter adenovirus in combination with adenovirus expressing siPdx-1 and endoplasmic reticulum Ca^{2+} was measured using Fluorescence Lifetime Imaging Microscopy (FLIM). A) Representative FLIM micrograph. B) Quantification of FLIM measurements. Indicated comparisons are significantly different (**$p < 0.01$) by Student's t-test. Results are displayed as the means ± S.E.M.

two-fold increase in SERCA2 protein levels (**Figure 11A-B**). Quantitative PCR also showed an increase in SERCA2b mRNA with exogenous Pdx-1 transduction (**Figure 11C**).

II.C.2. Pdx-1 Transduction Increases Transcription of a SERCA2 Luciferase Construct

In silico analysis demonstrated five putative Pdx-1 binding elements in the *SERCA2* promoter (**Figure 12A**). To determine if Pdx-1 was a transcriptional enhancer of the *SERCA2* gene, reporter assays were undertaken using different lengths of the human promoter fused to a luciferase coding gene. NIH-3T3 cells were co-transfected with *SERCA2* luciferase constructs and human Pdx-1 plasmid. Luciferase activity was measured 24 hours after transfection and normalized to total protein content. Co-transfection of Pdx-1 increased luciferase expression 3-4 fold over empty vector control in all constructs tested (**Figure 12B**). There were no significant differences between any of the constructs that were co-transfected with Pdx-1.

II.C.3. Pdx-1 Transduction Increases Transcription of a SERCA2 Luciferase Construct

All of the constructs included the most proximal putative site, so this suggested that the binding region closest to the transcriptional start site may be a key regulatory region for Pdx-1-mediated transcriptional enhancement of the *SERCA2* gene. This region of the *SERCA2* promoter maintains close homology between several species of mammals including human, mouse, and rat, and since it was a likely site for Pdx-1 binding, deletion-mutation of the proximal putative Pdx-1 element was undertaken (**Figure 13A**). Eight base pairs were deleted from the shortest length construct and no increase in

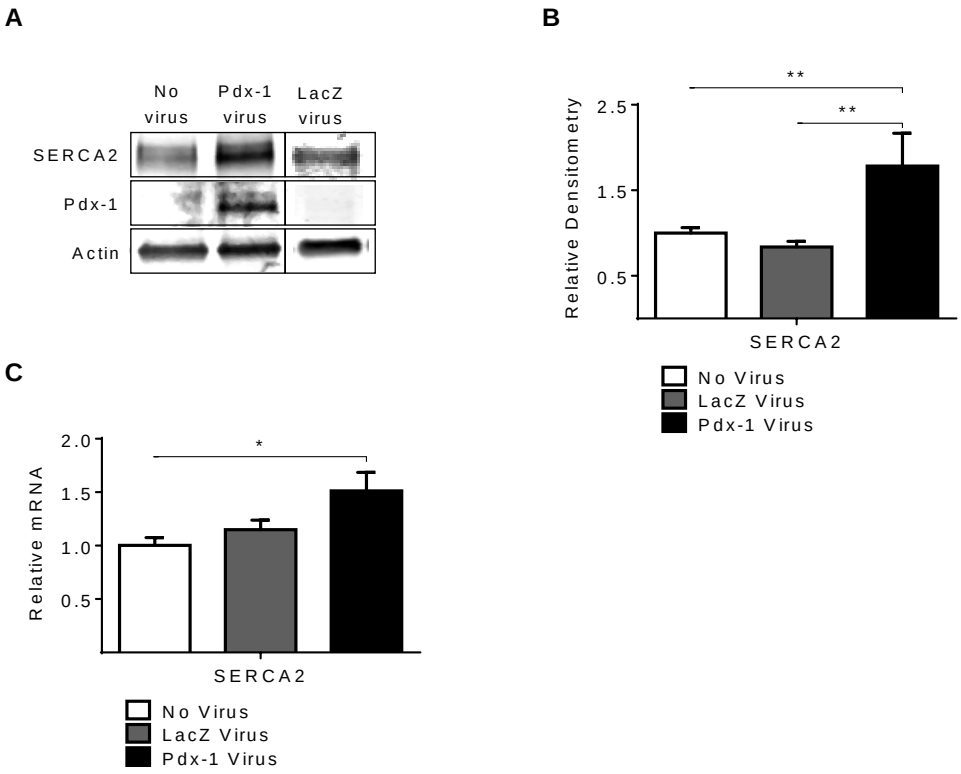


Figure 11. The effect of exogenous Pdx-1 overexpression on SERCA2b levels in NIH-3T3 cells. Pdx-1 was overexpressed via adenoviral transduction in NIH-3T3 mouse fibroblast cells that lack native expression of Pdx-1. A-B) Immunoblot was performed using antibodies against SERCA2, Pdx-1, and actin. A) Representative blot of Pdx-1 overexpression in NIH-3T3 cells. B) Quantification of immunoblot experiments. C) RNA was subjected to real-time PCR for quantification of SERCA2b transcript levels. Indicated comparisons are significantly different (*$p < 0.05$, **$p < 0.01$) by ANOVA with multiple comparisons. n=6 or greater. Results are displayed as the means ± S.E.M.

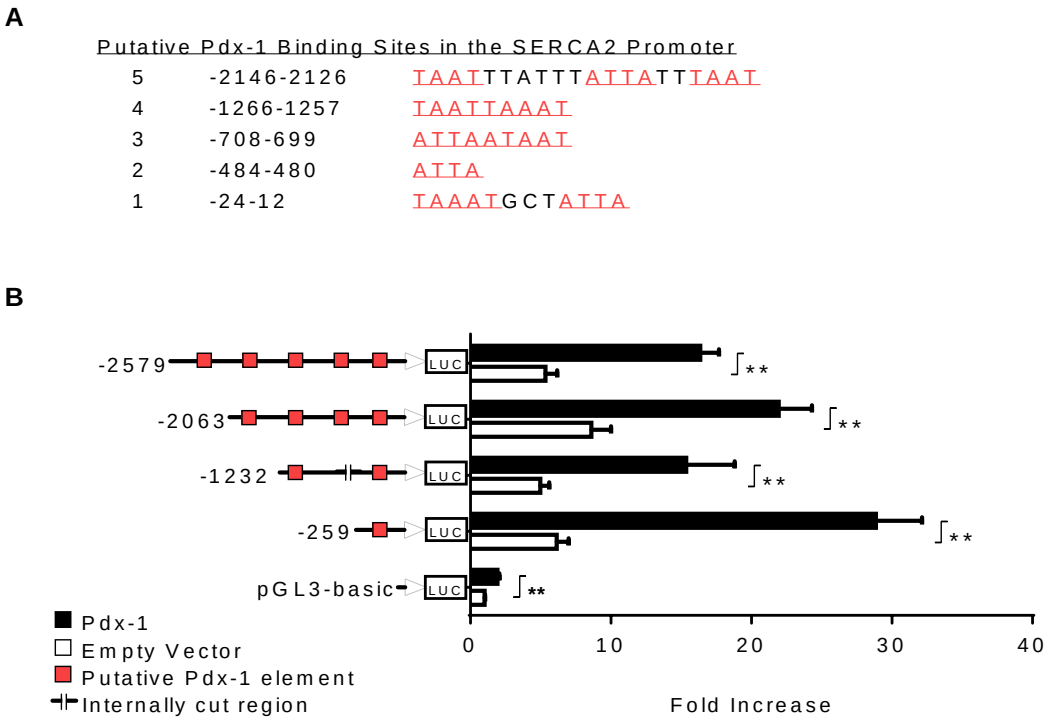


Figure 12. Co-transfection of Pdx-1 with *SERCA2* promoter luciferase constructs. Four constructs consisting of the human *SERCA2* promoter placed upstream of a luciferase coding region were transfected into NIH-3T3 mouse fibroblast cells. Luciferase activity was assayed comparing the results of Pdx-1 co-transfection to transfection with an empty vector control. A) Five putative Pdx-1 binding regions were identified in the human *SERCA2* promoter, each region containing between one and three potential binding sites. B) Results of co-transfection with Pdx-1 or with control for each *SERCA2* promoter construct. Indicated comparisons are significantly different (**$p < 0.01$) by multiple t-test with Sidak-Bonferroni post-test. n=10 or greater. Results are displayed as the means ± S.E.M.

luciferase activity was observed when co-transfecting Pdx-1 with this mutant construct (**Figure 13B**).

II.C.4. Pdx-1 Chromatin Immunoprecipitation of the *SERCA2* Promoter

For *in vivo* confirmation of direct Pdx-1 binding to the proximal Pdx-1 promoter region, chromatin immunoprecipitation (ChIP) experiments were performed using whole cell extract isolated from INS-1 cells. Results showed a 2-fold increase in recovery of the proximal *SERCA2* promoter following immunoprecipitation with anti-Pdx-1 antibody, and the confirmed Pdx-1 element of *INS1* promoter [337] was also enriched in the Pdx-1 immunoprecipitated sample (**Figure 14A**). The actin gene *ACTB* is not a target of Pdx-1 transcriptional enhancement, and it was not enriched in the immunoprecipitated sample, confirming that the pulldown of *SERCA2* and *INS1* promoter regions was not a generalized effect applicable to non-targets of Pdx-1 (**Figure 14B**).

II.D. Pdx-1 Haploinsufficient Mice Have Decreased Levels of SERCA2b

Complete knockout of Pdx-1 is perinatal lethal in mouse models, therefore Pdx-1 haploinsufficient mice were used to study the relationship between Pdx-1 and SERCA2b *in vivo* [344]. Mice were fed either a normal chow diet (17% of calories from fat) or a high fat diet (45% of calories from fat). The high fat diet was chosen to replicate the finding of Sachdeva et al. that islets from Pdx-1 haploinsufficient mice on high fat diet demonstrated ER stress [170].

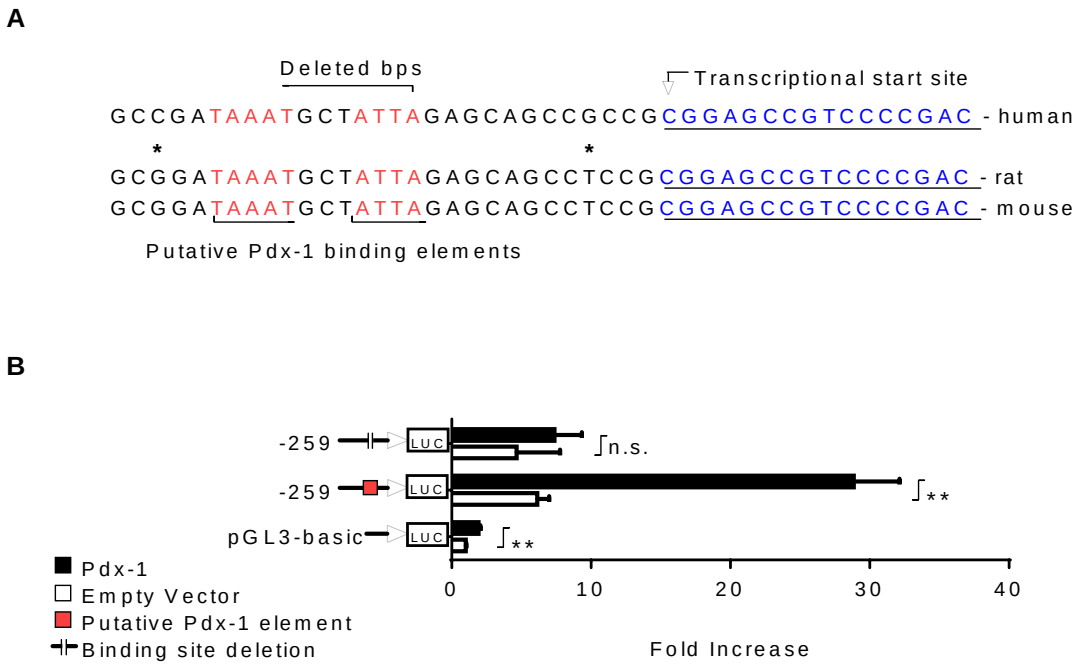


Figure 13. SERCA2 promoter luciferase construct with mutated proximal putative Pdx-1 binding element. To determine whether Pdx-1 enhanced *SERCA2* transcription at this site the proximal putative Pdx-1 was deleted and luciferase activity measured. A) Homology between human, rat, and mouse promoters is indicated. B) NIH-3T3 cells were co-transfected with wild-type or mutated *SERCA2* promoter and luciferase constructs combined with Pdx-1 plasmid or empty vector control. Indicated comparisons are either significantly different (**$p < 0.01$) or not significantly different ($^{n.s.}p > 0.05$) by multiple t-test with Sidak-Bonferroni post-test. n=5 or greater. Results are displayed as the means ± S.E.M.

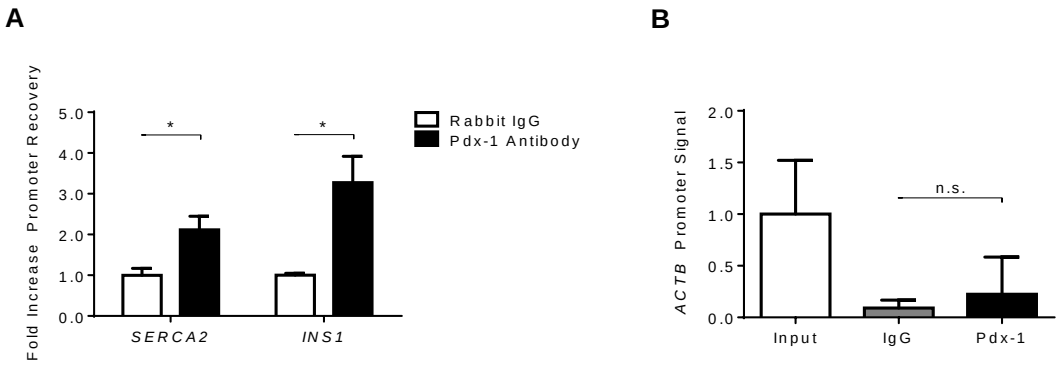


Figure 14. Pdx-1 directly binds the proximal SERCA2 promoter. INS-1 cells were harvested and subjected to chromatin immunoprecipitation (ChIP) analysis. Quantitative PCR was used to measure recovery of the *SERCA2* or *INS1* promoters following immunoprecipitation with anti-Pdx-1 antibody or normal rabbit IgG. A) Pulldown of the proximal putative Pdx-1 binding element in the *SERCA2* promoter and a confirmed Pdx-1 binding site in the *INS1* promoter region. Results are expressed as fold-increase in percent recovery of the target gene compared to rabbit IgG. B) Comparison of the Pdx-1 non-target *ACTB* promoter region between Pdx-1 antibody and normal rabbit IgG as compared to signal from the immunoprecipitation input sample. Results are expressed as fold-increase in percent recovery of the target gene compared to *ACTB* signal in the input sample. Indicated comparisons are significantly different (*$p < 0.05$) or not significantly different ($^{n.s.}p > 0.05$) by t-test, or by multiple t-test with Sidak-Bonferroni post-test. n=4 or greater. Results are displayed as the means ± S.E.M.

II.D.1. Pdx-1 Haploinsufficient Mice Fed Normal Chow Diet

Pdx-1 haploinsufficient mice and wild-type littermates were fed a normal chow diet containing 17% of calories from fat. At 11 weeks of age, intraperitoneal glucose tolerance tests (IPGTT) were performed. As previously observed by other groups, Pdx-1 haploinsufficient mice were glucose intolerant compared to wild type littermates (**Figure 15A-B**) [170, 271, 345, 346]. Although the Pdx-1 haploinsufficient mice were not frankly diabetic, SERCA2b protein and mRNA were significantly decreased in islets isolated from Pdx-1 haploinsufficient mice compared to wild type littermates (**Figure 15C-E**).

II.D.3. Pdx-1 Haploinsufficient Mice Fed High Fat Diet SERCA2b Expression

Pdx-1 haploinsufficient mice were fed a high-fat diet containing 45% calories from fat for 8 weeks, after which islets from Pdx-1 haploinsufficient and wild-type mice were isolated. Immunoblot analysis revealed that Pdx-1 and SERCA2 protein levels were decreased (**Figure 16A-B**). Isolated islets also showed a decrease in Pdx-1 and SERCA2b mRNA expression (**Figure 16C**). The X-box binding protein 1 (Xbp1) mRNA transcript becomes spliced to an active form when the β cell undergoes ER stress, and so both spliced and unspliced Xbp1 mRNA levels were measured in isolated islets. The ratio of spliced-to-unspliced Xbp1 was significantly higher in the islets of Pdx-1+/- mice fed high fat diets, providing evidence of ER stress (**Figure 16D**).

II.D.3. Reversal of ER Stress in Pdx-1 Haploinsufficient Mice with Restoration of SERCA2b Protein Levels

Isolated islets from high-fat diet fed Pdx-$1^{+/-}$ mice and wild type littermates were transduced with SERCA2b adenovirus or a LacZ control adenovirus. Successful transduction was verified by immunoblot (**Figure 17A**). With restoration of SERCA2b

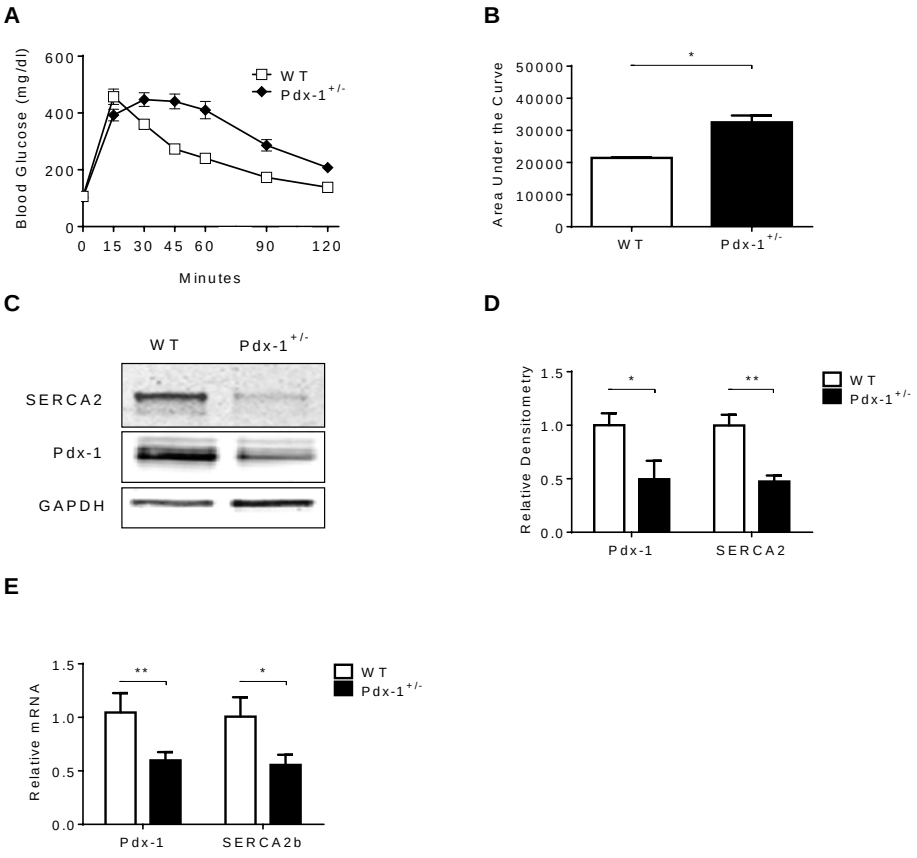


Figure 15. SERCA2b is decreased in islets isolated from Pdx-1 haploinsufficient mice. Pdx-1 haploinsufficient mice and wild type littermates fed a chow diet containing 17% of kilocalories from fat. A) Intraperitoneal glucose tolerance tests (IPGTT) were performed at 11 weeks of age in Pdx-1 haploinsufficient and wild type littermates. B) Area under the curve of IPGTT was quantified. C-E) Protein and RNA isolated from 13-week old Pdx-1 haploinsufficient and wild type littermate mouse islets. C) Representative immunoblot using antibodies against SERCA2, Pdx-1, and actin. D) Quantitative protein levels as measured by immunoblot. E) Islet mRNA measured by real-time PCR for quantification of SERCA2b and Pdx-1. Indicated comparisons are significantly different (*$p < 0.05$, **$p < 0.01$) by t-test or by multiple t-test with Sidak-Bonferroni post-test. Results are displayed as the means ± S.E.M.

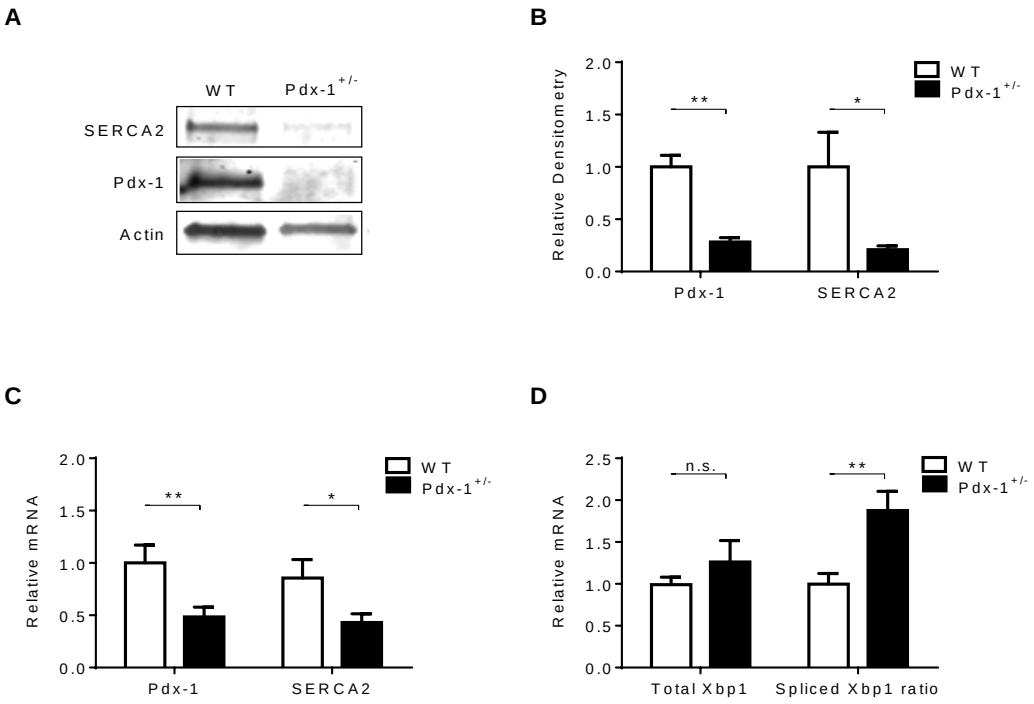


Figure 16. ER stress observed in islets isolated from diabetic Pdx-1 haploinsufficient mice fed a high fat diet. Pdx-1 haploinsufficient (Pdx-1$^{+/-}$) mice and wild type littermates were fed a high fat diet containing 45% kilocalories from fat for 8 weeks prior to islet isolation. A-B) Protein was isolated from 13-week old Pdx-1$^{+/-}$ and wild type littermate control mouse islets. A) Immunoblot was performed using antibodies against SERCA2, Pdx-1, and actin. B) Quantitative protein levels are shown graphically. C-D) RNA isolated from high fat diet fed Pdx-1$^{+/-}$ and wild type littermate mice was quantified by real-time PCR. C) Measurements of Pdx-1 and SERCA2b mRNA from isolated islets. D) Measurements of total and the ratio of spliced-to-total Xbp1 RNA from isolated islets. Indicated comparisons are significantly different (*$p < 0.05$, **$p < 0.01$) or not significant ($^{n.s.}p > 0.05$) by multiple t-tests with Sidak-Bonferroni post-test. Results are displayed as the means ± S.E.M.

expression in the islets of high-fat diet fed Pdx-$1^{+/-}$ mice, the ratio of spliced-to-unspliced Xbp1 was significantly reduced (**Figure 17B**).

II.E. PPARγ and Pdx-1 Bind the *SERCA2* Promoter in Close Proximity

Previous research in this laboratory has shown that there is a PPAR Response Element (PPRE) that allows PPARγ to bind the proximal region of the *SERCA2* promoter. This established PPRE is 13 nucleotides upstream from the Pdx-1 binding site investigated in the previously illustrated luciferase and chromatin immunoprecipitation experiments (**Figure 12B, 14A**). The proximal *SERCA2* promoter binding sites for Pdx-1 and PPARγ are in close proximity.

Other investigators have previously established that Serine-273 phosphorylation of PPARγ prevents enhancement of transcription in adipocytes but does not prevent binding of PPARγ to a target promoter. We hypothesized that steric hindrance arising from this post-translational modification of PPARγ may prevent the recruitment of Pdx-1 to the proximal SERCA2 promoter.

II.E.1. The PPARγ and Pdx-1 Binding Sites in the SERCA2 Promoter Are Situated in Close Proximity

The region of the *SERCA2* promoter containing both the established PPRE and the proximal Pdx-1 binding site has strong homology between human, rat and mouse species, and the two binding sites are also located near to one another along the linear DNA sequence of the *SERCA2* promoter (**Figure 18A**). The predicted crystal structure of Pdx-1 derived from a partial crystal resolution combined with structure prediction software is available, and when this predicted Pdx-1 structure is computationally docked with the established PPARγ structure eight of the ten most probable predicted docking

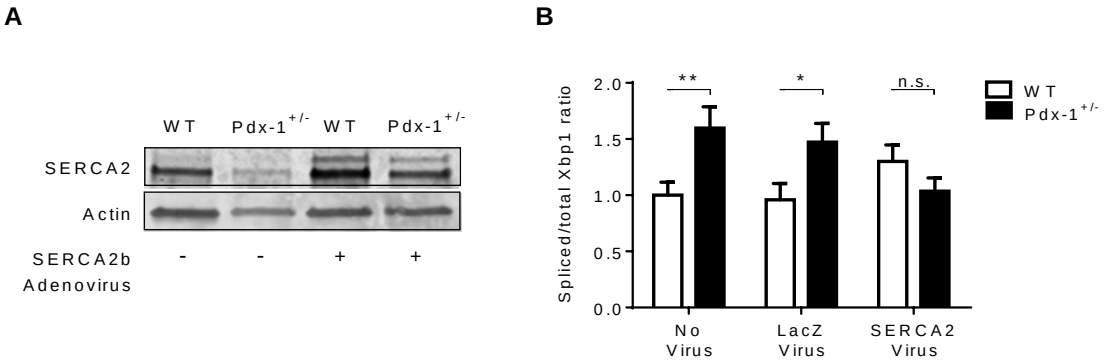


Figure 17. Reconstitution of SERCA2 expression ameliorates ER stress in islets isolated from Pdx-1 haploinsufficient mice fed a high fat diet. Pdx-$1^{+/-}$ mice and wild type littermates were fed a high fat diet containing 45% kilocalories from fat for 8 weeks prior to islet isolation. A) Immunoblot demonstrating successful adenoviral overexpression of SERCA2b in islets isolated from Pdx-$1^{+/-}$ mice and littermate controls fed high fat diet. B) Quantification of the ratio of spliced-to-unspliced Xbp1 mRNA in isolated islets from high fat diet fed Pdx-$1^{+/-}$ and control mice following SERCA2b adenoviral transduction, compared to no virus and to LacZ-expressing control virus. Indicated comparisons are significantly different (*$p < 0.05$, **$p < 0.01$). n=6 or greater. Results are displayed as the means $\pm$ S.E.M.

conformations place Pdx-1 adjacent to the Serine-273 phosphorylation site of PPARγ, one example of which is shown (**Figure 18B**).

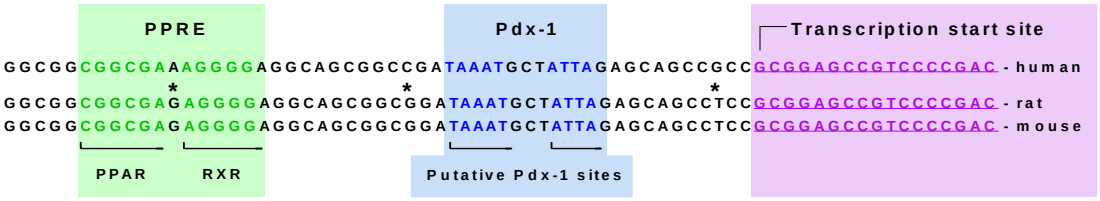
II.E.2 The PPARγ Agonist Pioglitazone Augments Pdx-1 Enhancement of *SERCA2* Promoter Activity

Previous work from this laboratory established that the thiazolidinedione drug pioglitazone increased transcription of *SERCA2* in the β cell [155]. If Pdx-1 and PPARγ cooperate at the *SERCA2* promoter, combining Pdx-1 transfection with pioglitazone treatment should result in increased luciferase signal from a *SERCA2*-luciferase construct containing both binding sites. In a preliminary experiment, cells transfected with both a *SERCA2*-luciferase reporter and Pdx-1 and treated with pioglitazone had increased luciferase activity compared to cells not treated with pioglitazone (**Figure 19A**).

II.F. Forkhead Box Protein 1 (FOXO1) Inhibits SERCA2 Transcription

In silico analysis of the *SERCA2* promoter also revealed a putative FOXO1 binding site 1333 base pairs (bp) upstream of the transcription start site (TSS) (**Figure 20A**). Although FOXO1 could inhibit *SERCA2* transcription by decreasing both Pdx-1 and PPARγ levels in the β cell [333, 335], the presence of a putative FOXO1 binding site in the *SERCA2* promoter suggests the possibility that inhibition of *SERCA2* transcription by FOXO1 might also be direct. When the full-length human *SERCA2*-luciferase construct was co-transfected into NIH-3T3 cultured mouse fibroblasts along with empty vector, FOXO1, Pdx-1 or a combination of these plasmids (**Figure 20B**), Pdx-1 alone increased luciferase activity as observed in prior experiments, however co-transfection with FOXO1 diminished this relative increase significantly. FOXO1 co-transfection did not result in a significant change in luciferase activity compared to empty vector control.

A



B

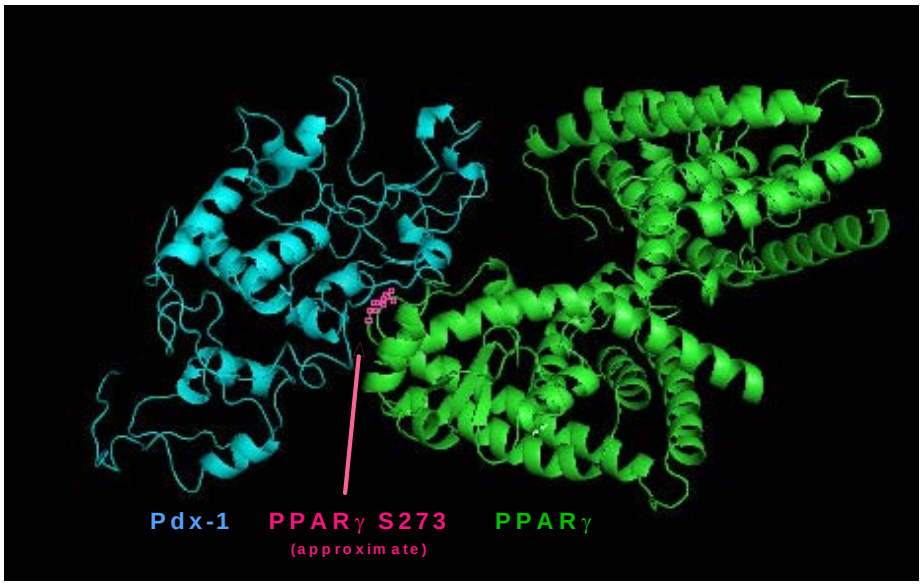


Figure 18. PPARγ and Pdx-1 binding sites in the proximal region of the SERCA2 promoter. The established PPRE in the *SERCA2* promoter is known to bind PPARγ, and the proximal putative Pdx-1 binding site in the *SERCA2* promoter is nearby. A) DNA sequence illustrating the proximal *SERCA2* promoter region and the respective PPARγ and Pdx-1 binding sites. B) Representative ribbon structure diagram illustrating one of ten probable docking configurations between Pdx-1 (cyan) and PPARγ (green) proteins, with an approximate location of the PPARγ phosphorylatable Serine-273 residue (fuchsia) highlighted.

A

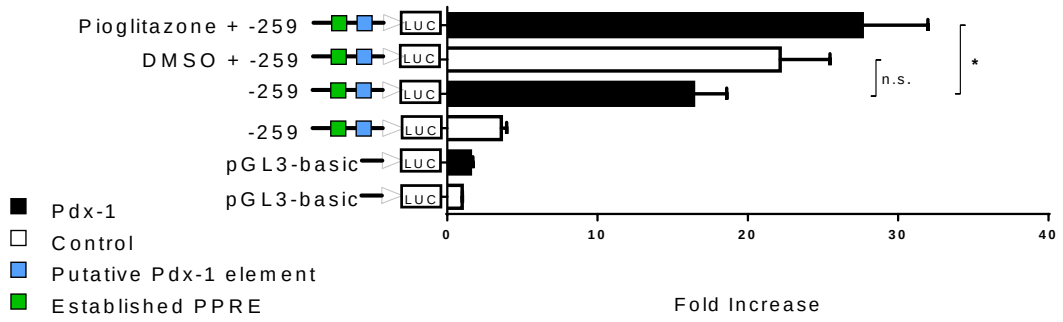


Figure 19. Luciferase reporter activity enhanced by Pdx-1 transfection, pioglitazone treatment. A) NIH-3T3 cells were co-transfected with a *SERCA2*-luciferase construct combined with Pdx-1 plasmid, and treated with pioglitazone. Indicated comparisons are significantly different (*$p < 0.05$) by multiple t-test with Sidak-Bonferroni post-test. n=4 or greater. Results are displayed as the means ± S.E.M.

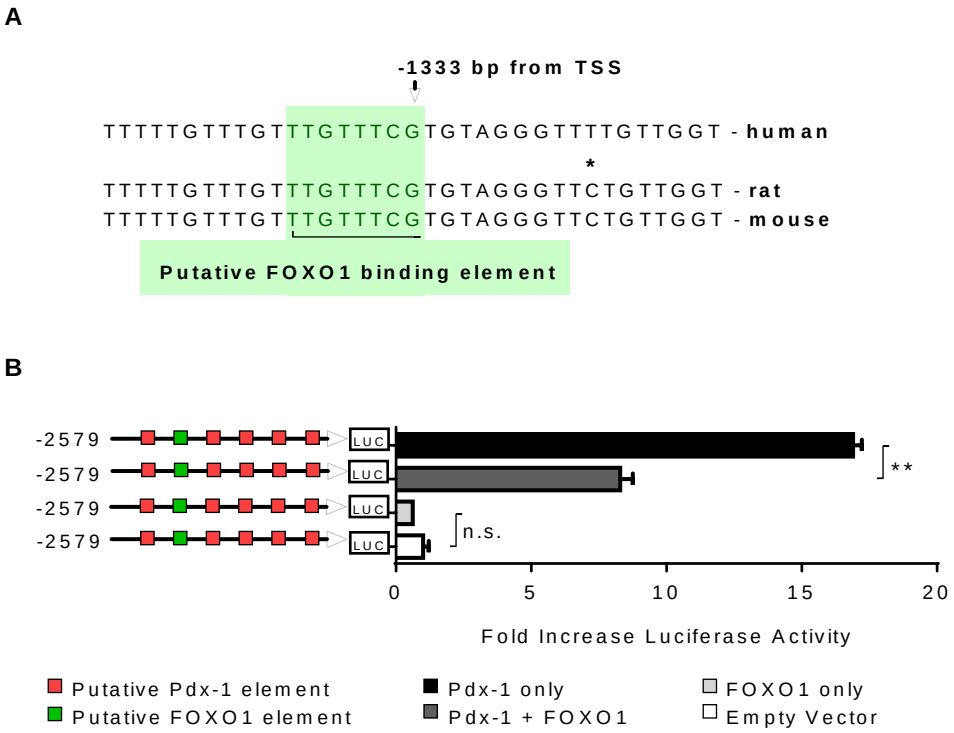


Figure 20. Luciferase reporter activity enhanced by Pdx-1 transfection, but enhancement decreased with FOXO1 co-transfection. A) Inter-species homology in the region of the putative FOXO1 binding site in the *SERCA2* promoter, B) NIH-3T3 cells were co-transfected with a *SERCA2*-luciferase construct combined with empty vector, FOXO1, Pdx-1 or a combination of those plasmids. Indicated comparisons are significantly different (**$p < 0.01$), or not significant ($^{n.s.}p > 0.05$) by one-way ANOVA with multiple comparisons. n=2 per condition. Results are displayed as the means ± S.E.M.

Chapter III. Discussion

A single β cell synthesizes approximately one million insulin molecules per minute - an arduous task that requires an extremely well-developed and highly functional ER [347]. ER stress in the pancreatic β cell is well appreciated in the context of obesity and Type 2 diabetes [348, 349], while more recent and emerging data suggest an expanding role for ER stress in the development and progression of Type 1 diabetes [136, 350, 351]. A key determinant of ER homeostasis is the maintenance of a robust intraluminal Ca^{2+} pool [157, 352]. This pool serves as an important store for Ca^{2+} release and activation of a variety of signaling pathways, including incretin-induced insulin secretion [353, 354]. Moreover, ER Ca^{2+} plays a pivotal role in protein processing and maturation through regulation of protein chaperone activity as well as the formation of chaperone complexes [157], while depletion of ER calcium inhibits protein synthesis and facilitates protein degradation [137-142]. Elegant studies have also illustrated a strict requirement for ER-derived Ca^{2+} in proinsulin processing in secretory granules, where Ca^{2+} is needed for maturation and activity of the endopeptidases prohormone convertase 1 (PC1) and 2 (PC2) [355, 356].

The SERCA family of ion pumps serves as a primary gatekeeper of this ER calcium gradient. In one catalytic cycle, SERCA transports two Ca^{2+} ions into the ER at the expense of one ATP molecule. At least three SERCA isoforms are known to be expressed in the pancreatic β cell; SERCA2a, 2b, and SERCA3. Whereas we have previously shown that expression of all three isoforms is decreased in islets isolated from diabetic rodents [201], this series of experiments was focused on the SERCA2b isoform as it is the most highly expressed isoform in mouse islets [357]. Furthermore, SERCA2b is structurally unique among all other isoforms because it possesses an extra transmembrane domain that imparts it with the highest calcium affinity [200]. Our previous work has shown that altered SERCA2b expression leads to altered insulin

secretion, activation of ER stress signaling, and decreased β cell survival [357]. The goal of this work was to identify additional transcriptional pathways that underlie dysregulated SERCA2b expression under inflammatory and diabetic conditions.

The homeobox protein Pdx-1 or IPF1 plays an essential role in pancreatic and β cell development, and the maintenance of postnatal β cell function, including transcriptional regulation of insulin gene transcription and other key genes involved in stimulus-secretion coupling [248, 271, 344]. Pdx-1 also plays a central role in β cell adaptation to metabolic stress, and Pdx-1 haploinsufficiency superimposed on a background of severe insulin resistance secondary to heterozygous deletion of the insulin receptor and insulin receptor substrate 1 leads to impaired compensatory β cell mass expansion, diabetes and premature mortality [271]. Sachdeva and colleagues examined the effects of diet-induced obesity in Pdx-$1^{+/-}$ mice and similarly found that Pdx-1 haploinsufficiency significantly limits β cell mass expansion under high-fat diet conditions. Interestingly, these effects were not secondary to decreased proliferation, but rather they were due to impaired β cell survival. In addition, isolated islets from HFD-fed Pdx-$1^{+/-}$ mice demonstrated activation of ER stress signaling pathways and similar results were seen in Pdx-1 deficient MIN6 cells. Microarray studies were performed in MIN6 cells transduced with adenovirally-expressed shRNA against Pdx-1 and revealed alterations in a large subset of genes with well-defined roles in the maintenance of ER function and UPR signaling, including Atf4, Wfs1, Ero1b, neuronatin (Nnat), and Heat shock 70kDa protein 5 (Hspa5/Bip). Pdx-1 was confirmed to directly bind the promoters of Atf4 and Wfs1, further confirming its role in maintenance of a specific β cell ER subgenome [170]. Alterations in the expression of Ero1lb and Nnat were confirmed in both Pdx-1 deficient MIN6 cells and islets isolated from Pdx-$1^{+/-}$ mice, although these genes were not identified as direct transcriptional targets [358, 359].

In addition to key ER-related genes previously identified, our results suggest that altered SERCA2b expression with concomitant alterations in ER Ca^{2+} contribute to ER stress observed in Pdx-1 deficient states. Here, we show that Pdx-1 and SERCA2b expression are altered in parallel in cadaveric human islets, in db/db islets and in an *in vitro* model of inflammatory diabetes. To test whether Pdx-1 directly regulates SERCA2b expression, overexpression and knockdown strategies were employed in NIH-3T3 and INS-1 cells, respectively. Results showed that Pdx-1 overexpression increases SERCA2b expression, while siRNA-mediated knockdown of Pdx-1 leads to a reciprocal reduction in SERCA2b. Luciferase assays and ChIP assays suggest this relationship is direct and show that Pdx-1 binds a proximal region of the human SERCA2 promoter. Importantly, we recapitulated the stress paradigm employed by Sachdeva et al. [170] and treated Pdx-$1^{+/-}$ mice with a high-fat diet with the goal of determining whether reconstitution of SERCA2b in islets *ex vivo* is sufficient to reverse ER stress secondary to Pdx-1 deficiency. Indeed, we show that restoration of SERCA2b expression is capable of mitigating ER stress, using spliced XBP-1 as a readout.

A novel aspect of our study is that we link modulation of Pdx-1 expression directly with alterations in β cell Ca^{2+} homeostasis. Specifically, using a genetically encoded and ER localized cameleon reporter [341], our results show that ER Ca^{2+} is decreased in a Pdx-1 deficient state. In parallel, cytosolic Ca^{2+} is increased, consistent with a deficiency in ER Ca^{2+} pump function. While our proposed model would suggest these changes are the result of alterations in SERCA2b expression and activity, previous literature has shown that Pdx-1 also enhances transcription of the *WFS1* gene. WFS1 is a transmembrane protein that has been localized to the β cell ER, plasma membrane, and secretory granules [360, 361]. Mutations in this gene lead to Wolfram or DIDMOAD syndrome in humans, a disorder characterized by childhood onset of diabetes, hypoinsulinemia, diabetes insipidus, optic atrophy, and deafness [362, 363], while single

nucleotide polymorphisms in Wfs1 have also been linked to T2D susceptibility in human populations [364]. Mouse models of diminished Wfs1 exhibit β cell apoptosis due to ER stress as well as decreased insulin secretion [365, 366]. The precise function of WFS1 in the β cell has remained somewhat enigmatic, though recently WFS1 was shown to play a role in cyclic AMP production, through translocation from the ER to the plasma membrane where it forms a complex with adenylyl cyclase 8 [361]. This protein has also been linked to the regulation of ER calcium, raising the possibility that a component of our calcium phenotype could be secondary to Wsf1 deficiency [367].

Notwithstanding this potential caveat, our results identify SERCA2b as a novel transcriptional target of Pdx-1 and identify an additional transcriptional pathway through which SERCA2b expression is altered in the β cell under diabetic conditions, and moreover demonstrate that ER Ca^{2+} regulation is altered in Pdx-1 deficient states.

Chapter IV. Relevance and Future Directions

IV.A. Novel Findings and Proposed Investigations

This work has demonstrated that Pdx-1 enhances SERCA2b transcription in the β cell. Previous work in the field has shown that diminished Pdx-1 results in β cell ER stress, and these results establish decreased β cell SERCA2b as a contributing mechanism for this observed ER stress. These results further provide evidence that restoration of SERCA2b expression in islets is sufficient to reverse ER stress due to Ca^{2+} dysregulation with Pdx-1 haploinsufficiency. These findings regarding Pdx-1 and SERCA2b are novel contributions to the field, and they also open further inquiries into the transcriptional regulation of SERCA2b in the β cell.

IV.A.1. Proposed Investigation: Pdx-1 and PPARγ Effect on SERCA2b

In silico analysis provided the clue that Pdx-1 might be a transcriptional enhancer of SERCA2b, but this analysis provided other clues as well. The proximal predicted binding site for Pdx-1 in the *SERCA2* promoter is located near an established PPAR response element (PPRE). These experimental results verified that Pdx-1 does bind the most proximal putative Pdx-1 binding element, which introduces the possibility of interaction between Pdx-1 and PPARγ at the *SERCA2* promoter. Investigating the interrelationship of PPARγ and Pdx-1 at the *SERCA2* promoter is a potential future direction of investigation.

IV.A.2. Proposed Investigation: Pdx-1 and FOXO1 Effect on SERCA2b

Another protein of interest suggested by *in silico* analysis of the *SERCA2* promoter is FOXO1, which is antagonistic to Pdx-1 in the β cell [334]. FOXO1 causes Pdx-1 to be exported from the nucleus and degraded in the cytosol [333], suggesting that FOXO1 might decrease *SERCA2* transcription. FOXO1 also inhibits PPARγ transcriptional

activity in the β cell [335]. If the putative FOXO1 binding site in the *SERCA2* promoter is valid, then direct binding of FOXO1 to the *SERCA2* promoter should inhibit *SERCA2* transcription.

IV.B. Co-localization of PPARγ and Pdx-1 at the *SERCA2* Promoter

Both PPARγ and Pdx-1 are known to form complexes to enhance transcription, though it has not previously been hypothesized that they cooperate with one another. If these two proteins do interact it may explain an unknown mechanism related to PPARγ function in the β cell. When PPARγ is phosphorylated at Serine-273 by CDK5 it is not prevented from binding the target gene promoters, but it does fail to enhance transcription of those genes [306], and this may also be the mechanism of PPARγ inhibition at the *SERCA2* promoter. If this phosphorylation causes steric hindrance it would prevent co-factors from also binding the promoter, and Pdx-1 could be one such co-factor inhibited by steric hindrance. Future studies will be designed to investigate whether PPARγ phosphorylation at Serine-273 inhibits recruitment and/or binding of Pdx-1 to the *SERCA2* promoter.

IV.B.1. Additional Pdx-1 Binding Sites in the *SERCA2* Promoter

The first step in determining whether Pdx-1 and PPARγ interact in the proximal region of the *SERCA2* promoter is to determine whether Pdx-1 binds at any other putative Pdx-1 binding sites. Although promoter analysis studies including ChIP and luciferase with both wild-type and mutated *SERCA2* promoter sequences indicated that the proximal site is bound by Pdx-1, these studies did not exclude any other putative Pdx-1 binding sites, of which there are four. If Pdx-1 binds any of the other putative sites then it would complicate, but not prevent, the study of the interaction between PPARγ and Pdx-1.

PPARγ-PHOSPHO-S273 STERIC HINDRANCE HYPOTHESIS

1) Determine all Pdx-1 binding sites in *SERCA2* promoter
   a) Luciferase mutations
   b) ChIP "promoter walking"

2) Models for PPARγ-Pdx-1 interaction experiments
   a) HG+IL-1β in vitro
   b) Pdx-$1^{+/-}$ mouse islets

3) Determine interaction
   a) Phosphomimetic $PPAR\gamma_{S273D}$
   b) Co-IP of Pdx-1 and PPARγ
   c) Luciferase with mutated constructs
   d) Islet transfection with $PPAR\gamma_{S273D}$

4) Alternatives
   a) Other diabetic mouse models (db/db, STZ)
   b) Other cultured cells, cytokines

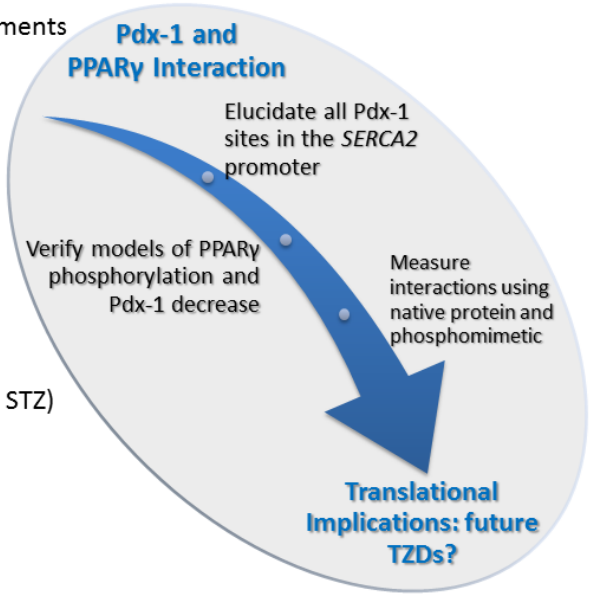


Figure 21. Experimental plan for future investigation of potential steric hindrance of Pdx-1 binding to the SERCA2 promoter due to PPARγ phosphorylation at Serine-273. The mechanism of inhibition via phosphorylation of PPARγ at Serine-273 in the β cell has not been established. We hypothesize that steric hindrance or electrostatic interference prevents formation of a transcriptional complex including Pdx-1 and PPARγ at the *SERCA2* promoter. We suggest that the listed experiments would provide evidence to support or refute this hypothesis.

IV.B.1.A. *SERCA2*-Luciferase Construct Mutation and Pdx-1

As an initial step to determine whether Pdx-1 binds other locations on the *SERCA2* promoter, additional mutation of a *SERCA2*-luciferase constructs would be necessary. If the proximal site were to be mutated on a *SERCA2*-luciferase construct containing all five elements, and during a luciferase experiment the enhancement of transcription was eliminated by mutation of the proximal promoter only, then that would suggest that the proximal site is the only relevant one.

IV.B.1.B. Pdx-1 Chromatin Immunoprecipitation

The initial ChIP experiment detailed in Results only tested for amplification of the most proximal region of the *SERCA2* promoter. by using qPCR primers that amplify incrementally more distal regions of the *SERCA2* promoter we would determine which regions can be precipitated with Pdx-1 antibody. If this “promoter walking” were to detect amplification of distal *SERCA2* promoter regions it would provide evidence that Pdx-1 binds additional putative Pdx-1 binding sites.

IV.B.1.C. Interpreting the Results

The proposed luciferase and promoter walking ChIP studies will provide evidence of whether Pdx-1 binds additional putative sites in the *SERCA2* promoter, and if Pdx-1 binds additional sites then the experimental plan would be adjusted to account for this.

IV.B.2. Models of Pdx-1 Decrease and PPARγ Phosphorylation at Serine-273

Prior to proceeding with further investigations of the interaction between Pdx-1 and PPARγ at the *SERCA2* promoter it would be necessary to establish *in vitro* and *in vivo* models of diabetic conditions. The foundation of any biological investigation must be a

thorough understanding of the models to be used, as well as proof that the experimental endpoints can be accurately and precisely measured.

IV.B.2.A. *In Vitro* Model of the β Cell in the Diabetic Milieu

This laboratory has previously cultured INS-1 832/13 rat insulinoma cells under in vitro conditions representing the hyperglycemic and inflammatory milieu of DM. Once again, we would culture INS-1 cells in 25 mM glucose and 5 ng/mL of IL-1β cytokine, expecting to observe a relative decrease in Pdx-1 levels and a relative increase in the phosphorylation of PPARγ Serine-273.

IV.B.2.B. *Ex Vivo* Model of the β Cell in the Diabetic Milieu

The Pdx-1 haploinsufficient mouse model is available, and the phenotype of this mouse model was previously described in the Results section. Islets isolated from both Pdx-$1^{+/-}$ and wild type littermates would be useful for investigations of the interaction between Pdx-1 and PPARγ. Treating of the islets *ex vivo* with high glucose and cytokines has previously been performed in this laboratory, and treating mouse islets with the PPARγ agonist pioglitazone has also been done previously [155]. Pdx-$1^{+/-}$ isolated islets would be treated with pioglitazone and SERCA2b expression levels compared to controls.

IV.B.2.C. Expected Results and Potential Challenges

Both INS-1 cells and islets would be treated with high glucose and cytokines to observe a decrease in Pdx-1 protein levels and an increase in PPARγ phosphorylation at Serine-273. The cells and islets would also be treated with pioglitazone and the effects on both Pdx-1 and PPARγ phosphorylation observed. It is expected that

treatment of Pdx-$1^{+/-}$ islets with pioglitazone would have decreased efficacy compared to islets from wild type mice.

If there were no differences, or if the differences were not as expected, then alternate models would be selected. For cultured cells MIN-6 mouse insulinoma cells could be substituted for INS-1 cells. A cytokine mix including IL-1β as well as interferon gamma and tumor necrosis factor alpha could be used instead of IL-1β alone. Other mouse models of DM could also be selected, including streptozotocin-treated (STZ) mice or db/db mice, where SERCA2b expression is similarly altered.

IV.B.3. Pdx-1 and PPARγ Phosphorylation at Serine-273

Once the *in vitro* and isolated islet models were established, and assuming a correlation between Pdx-1 and PPARγ was observed, the possibility of a direct relationship between Pdx-1 and PPARγ would be investigated. A critical tool for this aspect of the investigation would be a phosphomimetic version of PPARγ, wherein the Serine residue at the 273rd position would be mutated into Aspartate ($PPAR\gamma_{S273D}$). This phosphomimetic protein would be used in co-immunoprecipitation and luciferase studies.

IV.B.3.A. Co-Immunoprecipitation of Pdx-1 and PPARγ

As a first step, co-immunoprecipitation (Co-IP) of PPARγ and Pdx-1 would be pursued. Co-IP would be undertaken both with the native PPARγ protein and with the $PPAR\gamma_{S273D}$ phosphomimetic mutant.

IV.B.3.B. Luciferase Study With Pdx-1 and PPARγ

Preliminary experiments using the *SERCA2*-luciferase construct with pioglitazone and Pdx-1 have already been performed, and the results are suggestive of cooperation between these two factors. The preliminary experiments would be repeated, and the

PPARγ$_{S273D}$ phosphomimetic would also be used for additional luciferase studies. Additionally *SERCA2*-luciferase constructs with either the Pdx-1 or the PPARγ binding sites mutated would be used with either or both plasmids, and with the PPARγ$_{S273D}$ phosphomimetic. Fortunately PPARγ expression in NIH-3T3 mouse fibroblasts is relatively low [368] so the predominant PPARγ isoform would be the PPARγ$_{S273D}$ mutant.

IV.B.3.C. Mouse Islet Transduction with Pdx-1 and PPARγ$_{S273D}$ Phosphomimetic

Pdx-1 enhances transcription of *SERCA2*, therefore transduction of Pdx-1 into islets is expected to increase SERCA2b expression and reduce ER stress as measured by the spliced-to-unspliced Xbp1 mRNA ratio. Simply transducing the islets with Pdx-1 would not demonstrate cooperativity with PPARγ, however, so a further step must be undertaken. Islets would also be transduced with the PPARγ$_{S273D}$ phosphomimetic mutant as well as Pdx-1 adenovirus to more fully investigate the relationship between them.

IV.B.3.D. Expected Results and Potential Challenges

A successful Co-IP would provide evidence that the two proteins physically interact, and if the PPARγ$_{S273D}$ phosphomimetic failed to precipitate with Pdx-1 under the same conditions, this finding would support the steric hindrance hypothesis. If we did not succeed at co-precipitating Pdx-1 and PPARγ that would not be evidence that the two proteins do not interact, but rather it might simply signify that our precipitation conditions or our antibodies were not conducive to Pdx-1 and PPARγ interaction. Flag-tagged proteins could be used to attempt to address antibody concerns.

As for the luciferase experiments, preliminary results suggest that the effect of PPARγ and Pdx-1 are additive, however those experiments were done with pioglitazone and not PPARγ plasmid. If the PPARγ and Pdx-1 enhancement effects were found to be

multiplicative it would be evidence that the interaction between PPARγ and Pdx-1 is more important than either protein alone. The luciferase results obtained with the $PPAR\gamma_{S273D}$ phosphomimetic would be interesting in that if the phosphomimetic decreased the increase in luciferase signal from Pdx-1 then that would be evidence that Pdx-1 is subject to interference from PPARγ phosphorylation at Serine-273. If the $PPAR\gamma_{S273D}$ did not inhibit the Pdx-1 increase in luciferase construct with a mutated PPRE that would add further support to the steric hindrance hypothesis. Co-transduction of PPARγ and Pdx-1 should result in a greater increase of SERCA2 transcription than with either factor alone. If transduction of the $PPAR\gamma_{S273D}$ phosphomimetic mutant prevented or inhibited the increase of *SERCA2* transcription due to co-transduction of Pdx-1 that would provide evidence that the interaction of Pdx-1 and PPARγ was relevant to *SERCA2* transcription *in vivo*.

IV.C. Inducible PPARγ Serine-273-Aspartate Phosphomimetic Mouse Model

Mouse models heterozygous for PPARγ are available, as are mice with inducible Cre-Lox PPARγ deletion, however a mouse model representing inhibitory phosphorylation of PPARγ is not commercially available. Under stress conditions PPARγ is phosphorylated by CDK5 at the Serine-273 residue in the β cell, therefore an inducible $PPAR\gamma_{S273D}$ phosphomimetic mouse model could be used to investigate the role of PPARγ in β cell stress response mechanisms. include a transgenic construct with LoxP sites flanking the wild-type PPARγ gene as well as flanking a stop codon preventing transcription of the mutated $PPAR\gamma_{S273D}$ gene. Crossing this mouse with a Mouse Insulin Promoter and Cre Recombinase gene (MIP-Cre) would result in a β cell-specific inducible model of PPARγ pseudo-phosphorylation. Verification of induction would be via qPCR using primers specific to either wild type PPARγ or the $PPAR\gamma_{S273D}$ mutant.

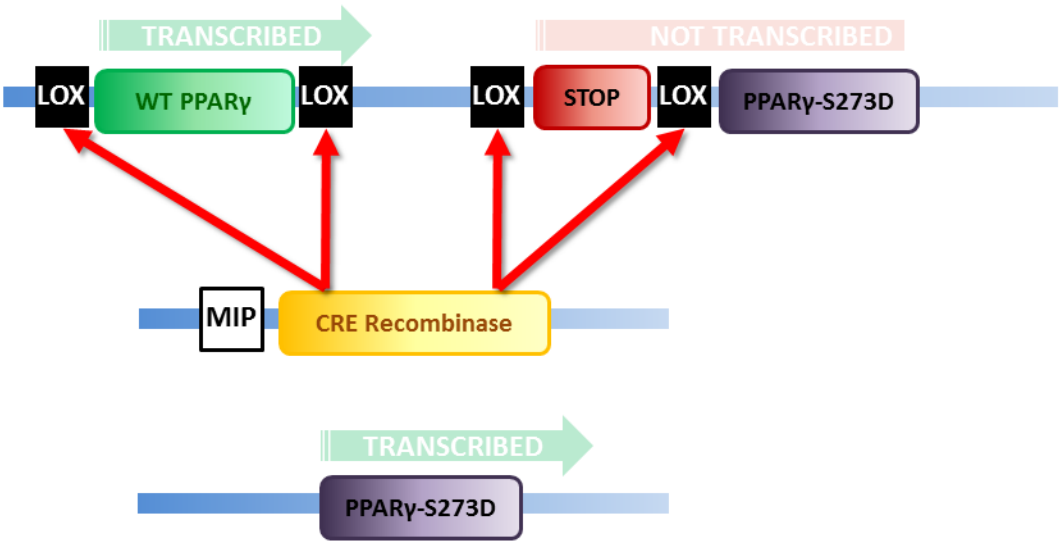


Figure 22. Mouse model of inducible phosphomimetic PPARγ via mutation of Serine-273 to Aspartate. The proposed mouse model of inducible phosphomimetic $PPAR\gamma_{S273D}$ would include Lox sites flanking a wild-type PPARγ gene as well as a stop codon preventing transcription of the mutated $PPAR\gamma_{S273D}$ gene. Crossing the mouse to a MIP-Cre line and then inducing Cre expression with tamoxifen would result in a β cell-specific inducible model of PPARγ pseudo-phosphorylation at amino acid 273.

MIP-Cre PPARγ$_{S273D}$ MOUSE MODEL

1) Characterize the mouse model phenotype
   a) Blood glucose measurements
   b) Glucose tolerance test
   c) Insulin tolerance test
   d) Islet and β cell morphology

2) Isolated islets
   a) Verification of induction via qPCR
   b) PPARγ, SERCA2b, Pdx-1 levels
   c) Spliced-to-unspliced Xbp1 ratio

3) Pdx-1 studies
   a) Adenoviral transduction
   b) Pdx-1 inducer drug
   c) Spliced Xbp1 ratio ER stress measurement

4) Alternatives
   a) HFD to induce glucose intolerant phenotype
   b) Cross to Pdx-1$^{+/-}$ line

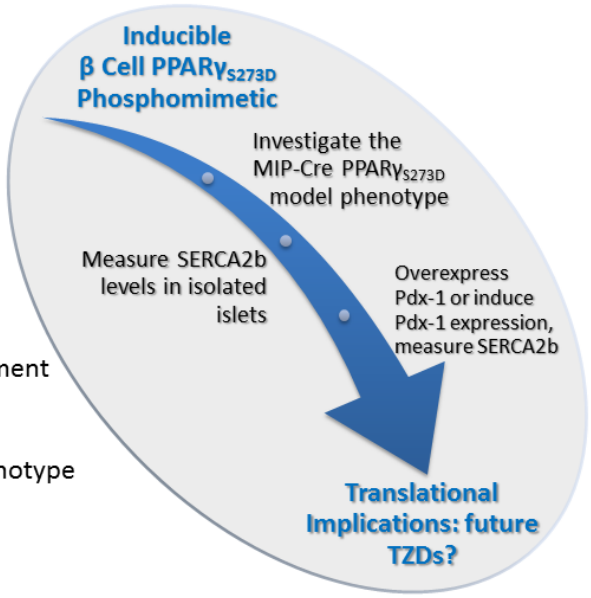


Figure 23. Characterization and investigations using the MIP-Cre PPARγ$_{S273D}$ mouse model. This MIP-Cre inducible mouse model would remove the wild type PPARγ and replace it with PPARγ$_{S273D}$ specifically in the β cell when treated with tamoxifen. First characterization of the overall metabolic phenotype would be required followed by quantification of Pdx-1, SERCA2b, PPARγ, and PPARγ$_{S273D}$ protein and mRNA. Isolated islets would then be transduced with Pdx-1 adenovirus or treated with the Pdx-1 inducer BRD7552 after which SERCA2b levels between PPARγ$_{S273D}$ and control islets would be compared.

IV.C.1. Phenotype of the MIP-Cre PPARγ$_{S273D}$ Mouse

Existing mouse models of PPARγ knockdown are homozygous lethal, and tissue-specific knockdown is also generally studied in heterozygotes [369]. Characterization of this new mouse model's metabolic phenotype would therefore compare controls to both heterozygous and homozygous inducible mutants. Random blood glucose measurement, glucose tolerance tests, and insulin tolerance tests would be used to test for diabetes, while islet histology would be used to observe and quantify islet and β cell mass. Those mice with the most diabetic phenotype would be considered optimal and would be used as models of β cell stress for subsequent experiments.

IV.C.2. Isolated Islets from the MIP-Cre PPARγ$_{S273D}$ Mouse

Pancreatic islets would be isolated from experimental and control mice, and qPCR primers designed to detect PPARγ$_{S273D}$ but not PPARγ would be used to verify induction. Protein immunoblot and qPCR would be used to measure Pdx-1, PPARγ, and SERCA2b protein and mRNA levels, while spliced and unspliced Xbp1 mRNA transcripts would be used to measure relative ER stress.

IV.C.3. Adenoviral Transduction of Islets from the MIP-Cre PPARγ$_{S273D}$ Mouse

Isolated islets from induced PPARγ$_{S273D}$ and control mice would be transduced with Pdx-1 adenovirus after which SERCA2b protein and mRNA levels would be measured and compared to controls. The ratio of spliced to unspliced Xbp1 would be used to quantify the extent of ER stress reduction with Pdx-1 transduction.

IV.C.4. Drug Treatment of Islets from the MIP-Cre PPARγ$_{S273D}$ Mouse

Pioglitazone and other TZDs are known agonists of PPARγ, however until recently small molecule agonists of Pdx-1 have not been available. The compound BRD7552

increases expression of Pdx-1 in β cells [370], and the effect of BRD7552 on SERCA2b expression and ER stress would be compared between PPARγ$_{S273D}$ and control islets. Isolated islets would also be subjected to *in vitro* high glucose and cytokine stress while being treated with either or both compounds, after which levels of PPARγ, Pdx-1 and SERCA2b protein and mRNA would be quantified and compared between PPARγ$_{S273D}$ and control islets.

IV.C.5. Expected Results and Alternatives

We expect that the PPARγ$_{S273D}$ mice will become glucose intolerant due to β cell defect, however if a suitable phenotype is not observed then the mice would be fed a diet with 45% of kilocalories from fat. An additional option would be to cross the MIP-Cre PPARγ$_{S273D}$ mice with Pdx-1 haploinsufficient mice to compare the compound phenotype.

We expect that islets isolated from PPARγ$_{S273D}$ mice will have decreased levels of Pdx-1 and SERCA2b protein and mRNA, since PPARγ is a transcriptional enhancer of both of those proteins. We further expect that the ratio of spliced-to-unspliced Xbp1 mRNA will be higher in PPARγ$_{S273D}$ islets. Treatment of PPARγ$_{S273D}$ islets with the Pdx-1 inducer BRD7552 should enhance transcription of SERCA2b in treated islets, however we predict that BRD7552 should have a decreased benefit in PPARγ$_{S273D}$ islets compared to controls.

Expression of other genes transcriptionally enhanced by PPARγ in the β cell would be measured via qPCR to determine the effect of the PPARγ$_{S273D}$ phosphomimetic on other proteins in the β cell. One gene directly enhanced by the PPARγ agonist is the insulin receptor substrate 1 gene *IRS1*, and additional genes that are secondarily enhanced are *INS1*, *INS2* and *GLUT2* [303]. If qPCR of these transcripts did not reveal a clear effect of PPARγ$_{S273D}$ then the methylation activity of SET-Domain Containing

Histone-Lysine N-Methyltransferases 7 and 9 could also be measured as a surrogate for relative PPARγ activity.

IV.D. FOXO1 and the *SERCA2* Promoter

In silico analysis predicted that Pdx-1 binds to the *SERCA2* promoter, and these results demonstrated that is indeed the case. In silico analysis also predicts that FOXO1 may bind the *SERCA2* promoter. If FOXO1 binds the *SERCA2* promoter and inhibits the transcription of *SERCA2* it might do so by two mechanisms; by direct transcriptional repression, or by indirect repression via downstream effectors, or even by a combination of the two.

IV.D.1. FOXO1 in Diabetic Models and Expected Results

Using the earlier established *in vitro* model of diabetic stress we would measure what effect hyperglycemic and inflammatory stress has on FOXO1 in cultured cells. FOXO1 protein and mRNA concentrations would be measured via immunoblot and qPCR, and cell fractions would also be differentially centrifuged in order to measure FOXO1 nuclear localization as a surrogate measure of FOXO1 activity.

IV.D.2. FOXO1 Overexpression and Knockdown

If FOXO1 is an antagonist of SERCA2b expression then overexpressing FOXO1 by viral transduction or plasmid transfection should decrease SERCA2b levels as measured by immunoblot or qPCR. Conversely, using siRNA versus FOXO1 should result in either an increase of SERCA2b in cultured cells, or amelioration of SERCA2b decrease under hyperglycemic and inflammatory conditions.

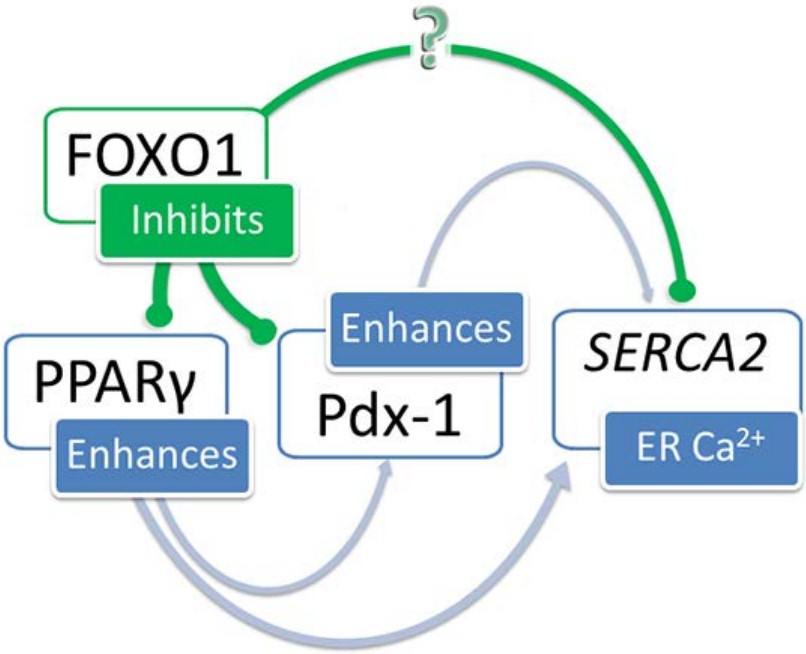


Figure 24. Interactions of Pdx-1, PPARγ and FOXO1 in the β cell. The transcription factor FOXO1 has long been established as an antagonist to Pdx-1, and recent evidence also suggests it is an antagonist to PPARγ in the β cell. The presence of a FOXO1 consensus binding sequence in the *SERCA2* promoter and the preliminary finding that FOXO1 impairs the enhancement of *SERCA2* transcription by Pdx-1 suggests that FOXO1 may bind the *SERCA2* promoter directly to inhibit *SERCA2* transcription.

FOXO1 INHIBITION OF *SERCA2* TRANSCRIPTION HYPOTHESIS

1) Determine effect of hyperglycemic and inflammatory stress on FOXO1
   a) *In vitro* model of HG+IL-1β
   b) Western blot, qPCR
   c) Cell fractionation/subcellular localization

2) FOXO1 overexpression and knockdown
   a) In cultured cells
   b) Western blot, qPCR

3) FOXO1 and *SERCA2* promoter analysis
   a) Luciferase with mutated construct
   b) ChIP with FOXO1 antibody

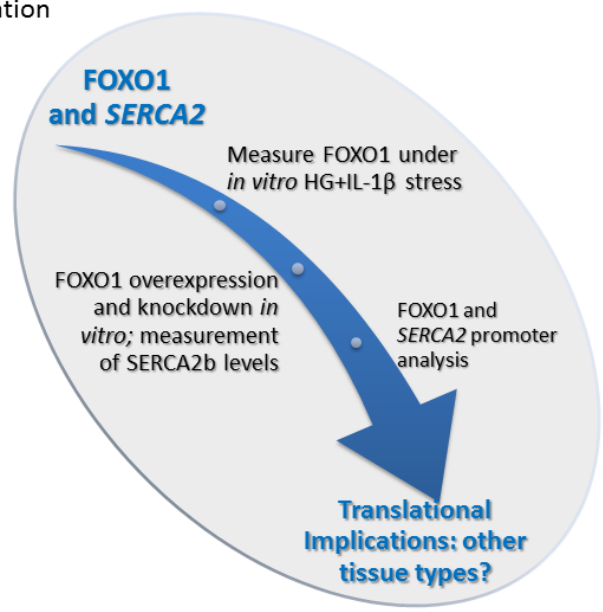


Figure 25. Experimental plan for future investigation of FOXO1 direct binding to the *SERCA2* promoter and inhibition of *SERCA2* transcription. FOXO1 inhibits both PPARγ and Pdx-1, which are both SERCA2b transcription enhancers in the β cell... In addition FOXO1 may have a direct inhibitory effect on *SERCA2* transcription by binding to the promoter. A series of *in vitro* and *ex vivo* tests would be carried out to determine the effect of FOXO1 on *SERCA2* transcription and whether that mechanism is direct, indirect or a combination of the two.

IV.D.3. FOXO1 and SERCA2 Promoter Analysis

Since FOXO1 inhibition of SERCA2b might be indirect instead of direct, promoter analysis is required to determine whether the mechanism of FOXO1 inhibition of SERCA2 transcription is via direct FOXO1 binding of the *SERCA2* promoter. The promoter analysis experiments used would be luciferase and ChIP.

IV.D.3.A. FOXO1 and *SERCA2*-Luciferase Constructs

Preliminary results transfecting FOXO1 plasmid with the *SERCA2*-luciferase construct demonstrated a significant reduction in luciferase expression. These results would be repeated and expanded upon. Although the shortest *SERCA2*-luciferase construct was suitable for experiments involving PPARγ, the putative FOXO1 site is distant from the transcription start site and therefore a longer construct would be used with FOXO1 alone or in concert with Pdx-1.

Mutated *SERCA2*-luciferase constructs could further elucidate the FOXO1 and SERCA2b relationship. A construct with a mutated FOXO1 binding site would be generated and tested for *SERCA2* promoter enhancement as measured by luciferase activity.

IV.D.3.B. FOXO1 and *SERCA2* ChIP

ChIP analysis of FOXO1 binding to the *SERCA2* promoter would provide direct evidence of the mechanism of FOXO1 suppression of SERCA2b expression. Primers for distal portions of the *SERCA2* promoter will have already been made and tested during the “promoter walking” process of testing for distal putative Pdx-1 binding sites.

IV.D.3.C. Expected Results

The preliminary luciferase experiments with FOXO1 were carried out in a cell type that did not natively express Pdx-1, suggesting that the inhibitory effect of FOXO1 on *SERCA2* transcription is not necessarily dependent on inhibiting Pdx-1. We expect that FOXO1 would continue to inhibit *SERCA2* transcription even in the presence of Pdx-1, but immunoblotting would be performed to determine whether the inhibition was due in whole or in part to degradation of Pdx-1 by FOXO1. If FOXO1 inhibition is able to overcome Pdx-1 enhancement of *SERCA2* transcription, we expect that whether or not the proximal Pdx-1 site was mutated this inhibition would be consistent, and if the mechanism of FOXO1 inhibition is via direct binding then mutation of its binding site should eliminate its inhibition effect on *SERCA2* transcription.

As for ChIP with FOXO1 and the *SERCA2* promoter, if FOXO1 antibody were to precipitate the distal portion of the *SERCA2* promoter it would provide further evidence that the binding of FOXO1 was direct, and that FOXO1 suppression of SERCA2b expression is not dependent only on suppression of Pdx-1 or other SERCA2b enhancing factors. ChIP might provide a false negative if FOXO1 is not located in the nucleus, so if the cells were stressed immediately prior to lysis that may increase binding of FOXO1 to the *SERCA2* promoter. Alternatively, exogenous FOXO1 could be transduced into cells prior to lysis. Finally, FOXO1 overexpression and knockdown mouse models are commercially available and could be used to study the effects of FOXO1 in isolated islets.

IV.E. Summary of Future Directions

ER Ca^{2+} homeostasis is a matter of life and death to the pancreatic β cell, therefore it should come as no surprise that SERCA2b expression is carefully managed by a number of transcription factors. The functional effects of SERCA2b regulation are now

becoming better understood, but the whole picture remains incomplete. What is the mechanism of PPARγ inhibition by phosphorylation at Serine-273? Does the otherwise pro-survival FOXO1 transcription factor downregulate SERCA2b expression in the β cell under hyperglycemic and inflammatory stress? The answers to these questions are not yet known, but understanding these mechanisms would further the field and potentially be applicable to other tissue types as well.

SERCA2a is also downregulated in cardiomyocytes after myocardial infarction, but the purpose and mechanism is not fully understood [371]. Restoration of SERCA function in cardiomyocytes is known to improve heart function, so increased understanding of factors capable of increasing *SERCA2* transcription may have translational applications not only for DM but also for heart failure [372]. Clinical trial results of a drug enhancing SERCA function in heart failure suggest that SERCA2 gene therapy may have efficacy improving cardiac function in patients recovering from cardiac events [373].

Lastly, it should be noted that there are ongoing efforts to design therapies to activate SERCA2a in myocytes in congestive heart failure including one drug istaroxime that has been recently studied in a Phase II trial . Calcium regulation also plays a role in cellular differentiation and de-differentiation in a variety of tissue types, including myocytes, osteoblasts and neurons [374-376]. Pdx-1 is the most essential transcription factor for differentiation of the pancreas from the embryonic foregut, and the extent to which this differentiation effect is dependent on Ca^{2+} regulation is unknown [247]. Furthermore, is the Ca^{2+} dysregulation observed due to a decrease in Pdx-1 and concomitant decrease in SERCA2b permissive for β cell dedifferentiation, and thus survival? Does Pdx-1 enhancement of SERCA2b contribute to an intracellular Ca^{2+} milieu favorable towards maintenance of mature β cell function? These questions have

not yet been explored, but the answers may provide increased understanding of pro-survival adaptations of the β cell under hyperglycemic and inflammatory stress.

As for a Cre-Lox model of $PPAR\gamma_{S273D}$ phosphomimetic mutation, it could potentially have broader application than the study of DM. PPARγ dysfunction is relevant to a plethora of diseases including insulin resistance but also lipodystrophy, hypertension, vascular nitric oxide signaling, and other diseases [369]. The $PPAR\gamma_{S273D}$ phosphomimetic mouse model could be used in many other studies in many tissue types, and thus it would be a novel mouse model with broad applicability across a host of systems to a number of diseases affecting human health.

For these reasons, and for potential translational applications, increased understanding of the effect of Pdx-1 and its cofactors or antagonists on *SERCA2* transcription is an important and novel contribution to the field of β cell biology and its role in diabetes mellitus.

Chapter V. Experimental Procedures

Chapter V.A. Animals and Islet Preparations

Animals were maintained under protocols approved by the Indiana University Institutional Animal Care and Use Committee, the U.S. Department of Agriculture's Animal Welfare Act (9 CFR Parts 1, 2, and 3), and the Guide for the Care and Use of Laboratory Animals [377]. C57BL/6J-db/db mice and heterozygote littermate controls were obtained from Jackson Laboratories (Bar Harbor, ME) at 12 weeks of age. PDX KO CC4 Pdx-1 haploinsufficient mice on a mixed background were a generous gift from Chris Wright at Vanderbilt University School of Medicine (Nashville, TN), and Pdx-1 haploinsufficient males were bred to C57BL/6J wild-type dams. Male offspring were weaned at age 4 weeks, and at age 5 weeks were either maintained on a normal chow diet (17% kcal from fat) or begun on high fat diet (45% kcal from fat) regiment for 8 weeks, to a total age of 13 weeks.

Cages were kept in a standard light-dark cycle with ad libitum access to food and water. Animals were housed in a secure facility on the premises of the Indiana University School of Medicine in plastic cages with sterilized corn-cob bedding. No more than five mice and no less than two mice were housed in any cage. Bedding was changed at weaning, every 14 days, or after intraperitoneal glucose tolerance testing (IPGTT).

To determine the Pdx-1 haploinsufficiency genotype, male offspring of PDX KO CC4 sires were genotyped for the presence of the CC4 gene using a PCR protocol and primers obtained from the laboratory of Dr. Chris Wright. Because of the possibility of false negatives due to insufficient or low-quality DNA, a separate genotype for the SET7/9 gene was run as a positive control. The SET7/9 genotype protocol was chosen because this laboratory has previously found it to be robust. Genotype test results were counted as accurate if a band for the SET7/9 gene was present, and mice were counted as Pdx-1 heterozygotes if a band corresponding to the CC4 gene was detected.

IPGTT were performed at 11 total weeks of age using a previously described protocol [378, 379]. Briefly, mice were fasted 14 hours overnight, and the following morning each mouse was weighed while the initial time-zero blood glucose measurement taken. Each individual animal was then injected intraperitoneally with a sterile glucose solution corresponding to the mouse's weight. Blood glucose measurements were taken 15, 30, 45, 60, 90 and 120 minutes after injection.

Random blood glucose was measured in the initial experimental cohort from total age 8 weeks to total age 13 weeks to verify a non-diabetic phenotype. For both random blood glucose measurements and IPGTT, blood glucose concentration was measured using an AlphaTRAK glucometer from Abbott Laboratories (Abbott Park, IL) using blood from a small incision in the tail vein.

Chapter V.B. Cell Culture and *In Vitro* Adenoviral Transduction

Immortalized cultured cells were used for *in vitro* experiments, with rat insulinoma cells used to in experiments requiring Pdx-1, and mouse fibroblast cells used in experiments where endogenous Pdx-1 was undesirable for experimental design purposes.

INS-1 832/13 rat insulinoma cells were cultured as previously described [201, 380]. The INS-1 cells were cultured at 37ºC with 5% CO2 in Roswell Park Memorial Institute media 1640 containing 11.1 mM glucose supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, and 50 µM β-mercaptoethanol. When split the cells were trypsinized and replated for experimental or passage purposes. INS-1 cells were passaged using 12 mL of culture media in T-75 flasks, and media was replaced every 24-48 hours. Prior to use in experiments INS-1 cells were counted by hemocytometer before plating in cell-

culture coated 12-well or 6-well plates, with approximately $1.5x10^5$ or $3x10^5$ cells plated per well in 12-well and 6-well plates, respectively.

NIH-3T3 immortalized mouse fibroblast cells were cultured at 37ºC and 5% CO_2 in Dulbecco's Modified Essential Medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin [381]. When split the cells were trypsinized and replated for either experimental or passage purposes. NIH-3T3 cells were passaged in 12 mL of media in T-75 flasks with the media on passaged cells replaced every 24-48 hours. When used for experiments, NIH-3T3 cells were counted by hemocytometer prior to plating in cell-culture coated 12-well or 6-well plates, with between $7.5x10^4$ and $1.5x10^5$ cells plated per well in 12-well and 6-well plates, respectively.

This laboratory has previously published an in vitro model of treating cells with 25 mM glucose and 5ng/mL IL-1β for 24 to test hyperglycemic and inflammatory conditions [155]. Briefly, INS-1 cells were split approximately 1.5 x105 cells per well in a 12-well coated culture dish. The following day 9 parts fresh INS-1 media (see cell culture section) was mixed with 1 part INS-1 media with 151 mM glucose concentration. To this high glucose media was added 5 ng/mL of mouse IL-1b inflammatory cytokine then the mixed HG+IL-1b media was placed onto the INS-1 cells in lieu of normal media. Cells were then cultured for either 16 or 24 hours at 5% CO2 and 37C prior to rinsing with PBS and lysis for either quantitative PCR or Western protein immunoblot.

For adenoviral transduction in cell lines, culture media was replaced with fresh media containing an adenovirus expressing either hamster Pdx-1, mouse SERCA2b [382], Pdx-1 siRNA [342], random siRNA [383], or a LacZ control virus, followed by 24 hours incubation. Interfering RNA sequences used were as follows: siPdx-1: 5'-GAAAGAGGAAGATAAGAAA-3'; random siRNA: 5'- GAGACCCTATCCGTGATTA-3'. For each experiment approximately equivalent concentration of virus was added for either

experimental or control conditions. After 24 hours, RNA or protein was isolated from transduced cells for indicated analyses.

Chapter V.C. Isolated Islets and Adenoviral Islet Transduction

Mice were 13 weeks of age at islet isolation. Islets were isolated by technicians in the islet core facility of the Wells Center Diabetes Research Group, using collagenase digestion as described previously [384]. Primary islets were suspended in RPMI media after isolation, then placed in 6-well uncoated culture dishes and incubated at 37C with 5% supplemental CO_2 for approximately one hour. Islets were visually counted and then pooled by genotype. For protein immunoblot approximately 100 islets were selected for lysis and purification, and for mRNA isolation approximately 50 islets were selected for lysis and purification.

If islets were to be virally transduced, prior to lysis they were placed into wells of a new uncoated culture plate, suspended in Dulbecco's Modified Essential Medium including 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin, and incubated with either mouse SERCA2b or LacZ adenovirus for 24 hours, with lysis and purification performed the following day. Islets were treated with 5 times the concentration used for cultured cells.

Human islets were obtained from NIDCC. Our analysis included islets from five nondiabetic donors and three donors with a previous diagnosis of T2D. The average donor age was 48.1 $\pm$ 4.2 years (S.E.M.), and the average body mass index (BMI) was 25.8 kg/m2 $\pm$ 2.1 (S.E.M.). Upon receipt, cadaveric human islets were placed at 37C and 5% CO2 and allowed to acclimate for 24 hours. The following day islets were counted and lysed for mRNA isolation in the same manner as mouse islets.

Chapter V.D. Protein Immunoblot and Analysis

Isolated islets or cultured cells were washed with PBS and lysed with buffer containing 50mM Tris (pH 8.0), 150 mM NaCl, 0.05% Desoxycholate, 0.1% IGEPAL CA-630 (Sigma-Aldrich, St. Louis, MO), 0.1% SDS, 0.2% sarcosyl, 10% glycerol, 1mM DTT, 1mM EDTA, 10mM NaF, EDTA-free “Complete Mini” protease inhibitors (Roche Applied Science, Penzburg, Germany), “PhosphoStop” phosphatase inhibitors (Roche Applied Science), 2mM $MgCl_2$, and 0.05% v/v benzonase nuclease (Sigma-Aldrich). Lysate from isolated islets was further disrupted by mechanical shearing using a 20 gauge needle and syringe.

Protein concentration was measured using the Bio-Rad DC protein assay (Bio-Rad, Hercules, CA) and a SpectraMax M5 multiwell plate reader (Molecular Devices, Sunnyvale, CA). Equal concentrations of proteins were suspended in 10% SDS solution and heated to 70C for 5 minutes to more fully denature the proteins prior to electrophoresis using a 4-20% Mini-Protean TGX gel in a Mini-Protean Tetra apparatus (Bio-Rad). Protein lysates were electrophoresed at room temperature applying 90 volts for 90 minutes from a constant voltage power supply. The separated proteins were transferred to methanol-activated PVDF membrane at 90 volts for 60 minutes at approximately 4C using a constant voltage power supply. Membranes were then protein blocked with Odyssey blocking buffer (LI-COR, Lincoln, NE) prior to incubation with primary antibodies (**Table 1**). Dried immunoblot membranes were scanned using a LI-COR Odyssey 1828 scanner and analyzed with LI-COR Image Studio software (LI-COR), with densitometry of scanned images calculated by Image-J software (National Institutes of Health, Bethesda, MD). Membranes were re-wetted with methanol and PBST prior to incubation with a second or third detection antibody.

TABLE 1. Immunoblot antibodies and incubation conditions			
Pdx-1	Millipore	07-696	40 hours, 4C, Shaker table
SERCA2	Santa Cruz	sc-8095	40 hours, 4C, Shaker table
Actin	MP Biomed	691002	2 hours, 23C, Shaker table
GAPDH	Abcam	ab9484	2 hours, 23C, Shaker table

Table 1. Primary and secondary antibodies used for immunoblot. The proteins of interest were Pdx-1 and SERCA2b with actin as a loading control for most experiments, with GAPDH used as an alternative loading control for *ex vivo* immunoblot of isolated mouse islets. All antibodies had cross-reactivity for all human, rat and mouse tissues. The SERCA2b antibody used is not capable of distinguishing between SERCA2 isoforms.

Chapter V.E. Quantitative Real Time PCR (qPCR)

Cultured cells or isolated islets were processed for total RNA using RNeasy Mini plus or Micro plus kits (Qiagen, Valencia, CA), according to manufacturer's instructions and as described previously [385, 386]. For quantitative RT-PCR experiments, total RNA was reverse-transcribed at 37°C for 1 hour using 15 µg random hexamers, 0.5 mM dNTPs, 5X first-strand buffer, 0.01 mM DTT, and 200 units of Moloney murine leukemia virus (MMLV) reverse transcriptase (all from Invitrogen, Grand Island, NY). Quantitative RT-PCR was performed as described previously using either SYBR Green I dye and previously published primer sequences [342, 386-388], or using TaqMan proprietary primers (Life Technologies, Grand Island, NY) (**Table 2**).

Chapter V.F. Fura-2 AM Cytoplasmic Calcium Imaging

Approximately 5 x10^5 INS-1 832/13 cells were seeded in a glass bottom 50 mm plate 24 hours prior to transduction with siPdx-1 adenovirus, random siRNA adenovirus, or no adenovirus for an additional 20 hours. The cells were then incubated at 37ºC plus 5% CO_2 in GIBCO Hank's Balanced Salt Solution (Life Technologies, Grand Island, NY) plus Fura-2 AM fluorescent dye (Life Technologies) for one hour. After Fura-2 AM incubation, cells were placed in HBSS without Fura-2 AM and maintained at 37ºC plus 5% CO_2 during imaging. Random cells in a random field were selected and imaged for fluorescence at 340 and 380 nm. Images were captured using a Zeiss Z1 microscope with a 20x objective (Zeiss, Oberkochen, Germany), and results were analyzed with Zen Blue software (Zeiss).

Chapter V.G. Fluorescence Lifetime Imaging Microscopy (FLIM)

A D4ER adenovirus that has been previously published was used for spectrometric analysis of ER Ca^{2+} [341]. This probe was created by replacing the Ca^{2+} binding domain

TABLE 2. Quantitative PCR primers and temperatures		
SERCA2b (mouse)	Forward: 5'-GATCCTCTACGTGGAACCTTTG-3' Reverse: 5'-CCACAGGGAGCAGGAAGAT-3'	1x 95C 10 minutes, 65C 1 minute; 40x 65C 1 minute, 60C 30 seconds, 95C 30 seconds
SERCA2b (rat)	Forward: 5'-GTGGAACCTTTGCCACTCAT-3' Reverse: 5'-TGTGCTGTAGACCCAGACCA-3'	1x 95C 10 minutes, 65C 1 minute; 40x 65C 1 minute, 60C 30 seconds, 95C 30 seconds
SERCA2b (human)	Forward: 5'-ACAATGGCGCTCTCTGTTCT-3' Reverse: 5'-ATCACCAGGGGCATTATGAG-3'	1x 95C 10 minutes, 65C 1 minute; 40x 65C 1 minute, 60C 30 seconds, 95C 30 seconds
Actin (mouse, rat, human)	Forward: 5'-AGGTCATCACTATTGGCAACGA-3' Reverse: 5'-CACTTCATGATGGAATTGAATGTAGTT-3'	1x 95C 10 minutes, 65C 1 minute; 40x 65C 1 minute, 60C 30 seconds, 95C 30 seconds
Total Xbp1 (mouse)	Forward: 5'- TGGCCGGGTCTGCTGAGTCCG-3' Reverse: 5'- GTCCATGGGAAGATGTTCTGG-3'	1x 95C 10 minutes, 65C 1 minute; 40x 65C 1 minute, 60C 30 seconds, 95C 30 seconds
Spliced Xbp1 (mouse)	Forward: 5'- CTGAGTCCGAATCAGGTGCAG-3' Reverse: 5'- GTCCATGGGAAGATGTTCTGG-3'	1x 95C 10 minutes, 65C 1 minute; 40x 65C 1 minute, 60C 30 seconds, 95C 30 seconds
Pdx-1 (mouse)	Taqman: Mm00435565_m1	1x 95C 10 minutes, 65C 1 minute; 40x 65C 1 minute, 95C 30 seconds
Pdx-1 (rat)	Taqman: Rn00755591_m1	1x 95C 10 minutes, 65C 1 minute; 40x 65C 1 minute, 95C 30 seconds
Pdx-1 (human)	Taqman: Hs00236830_m1	1x 95C 10 minutes, 65C 1 minute; 40x 65C 1 minute, 95C 30 seconds

Table 2. Quantitative PCR analysis of messenger RNA. Primers used for mRNA analysis in mouse and rat cultured cells, as well as in human and mouse islets. SYBR green was used for all mRNA measurements except for measurement of Pdx-1 mRNA, which used the proprietary TaqMan combined primer and dye in all three species.

of the D1ER construct with D4, to provide lower Ca^{2+} affinity and placing it downstream of the rat insulin promoter. Briefly, INS-1 cells were cultured for 18 hours with the D4ER adenovirus and siPdx-1 adenovirus or random siRNA adenovirus and then allowed to recover for 6 hours. Fluorescence Lifetime Imaging Microscopy (FLIM), was carried out in accordance with a previously published protocol [389-391]. The Alba FastFLIM system (ISS Inc., Champaign, IL) was coupled to an Olympus IX71 microscope using 60x water-immersion lens (Olympus, Tokyo, Japan). Confocal scanning was controlled by Build 143 VistaVision software (ISS Inc.) at 530/43 nm acceptor and 480/40 nm receptor wavelengths. Regions of interest were selected with typically >75 count average. Efficiency of Förster Resonance Energy Transfer (FRET) was estimated by the following equation: $EFRET=1-T_{DA}/T_D$. Averaged results per cell were plotted as a function of accepted-to-donor ratio (I_A/I_D).

Chapter V.H. Luciferase Reporter Assay

A previous publication from this laboratory utilized luciferase constructs incorporating different lengths of the human SERCA2 promoter [155], and the same constructs were used for this series of experiments. Approximately 2.0 $x10^4$ NIH-3T3 mouse fibroblast cells were seeded in 12-well plates 24 hours before transfection. Approximately 200 µg of plasmid was transfected into cells using Metafectene Pro transfection reagent (Biotex, Munich, Germany) according to manufacturer instructions. During transduction, cells remained in GIBCO Opti-MEM transfection media (Life Technologies) for 6 hours, followed by incubation for 18 additional hours in normal media and then lysis.

Total luminescence was measured in triplicate using the Promega Luciferase Assay System kit (Promega, Madison, WI) using a SpectraMax M5 plate reader (Molecular Devices) in top-read mode of 3000 ms per well. Luminosity results were normalized to total protein content as measured with the same BCA assay technique described for

immunoblotting (Bio-Rad). A luciferase construct with a deleted putative proximal Pdx-1 binding site was created by mutagenesis using the Stratagene QuickChange Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer's instructions, and the site-specific deletion was confirmed by automated DNA sequencing (GENEWIZ, South Plainfield, NJ).

Chapter V.I. Chromatin Immunoprecipitation (ChIP)

Approximately 3.25 x10^6 INS-1 cells were fixed in 1% formaldehyde for 10 minutes, sonicated to shear DNA fragments into the size range of 800-2000 bp, and then subjected to chromatin immunoprecipitation (ChIP) as detailed previously [155, 387]. Crosslinked protein and promoters were incubated for approximately 35 hours under nutation at 4^{o}C using anti-Pdx-1 antibody sc-14664X (Santa Cruz Biotechnology, Dallas, TX) with normal rabbit immunoglobulin G (Santa Cruz Biotechnology) as a control. Samples were quantitated in triplicate by SYBR Green I based quantitative real-time PCR as previously described [386] using forward and reverse primer sequences for the rat SERCA2 promoter with the following sequences: Forward 5'-CGCTTTTGGCTGTGTGGGAAG-3', Reverse 5'-TGGTGTCCTTGGCTTGCCTC-3', the rat insulin 1 (INS1) promoter with the following sequences: Forward 5'-TCAGCCAAAGATGAAGAAGGTCTC-3', Reverse 5'-GCATTTTCCACATCATTCCCC-3'.

Chapter V.J. Statistical Analysis

Differences between groups were analyzed for significance using unpaired Student's t-Test, one-way ANOVA with multiple comparisons and Tukey-Kramer post-test, Best-fit analysis, or multiple t-Tests with Sidak-Bonferroni correction [392], as calculated by GraphPad Prism 6.01 statistics software. A p value < 0.05 was considered to indicate a significant difference between groups.

References

1. Centers For Disease Control, (2011) National Diabetes Fact Sheet. http://www.cdc.gov/Diabetes/, Accessed 6/5/2014, Atlanta, GA, U.S.A.
2. Ashcroft, F. M., and Rorsman, P. (2012) Diabetes Mellitus and the Beta Cell: The Last Ten Years. *Cell* **148**, 1160-1171
3. Alberti, K. G., and Zimmet, P. Z. (1998) Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications. Part 1: Diagnosis and Classification of Diabetes Mellitus Provisional Report of a WHO Consultation. *Diabetic Medicine: A Journal of the British Diabetic Association* **15**, 539-553
4. Cooke, D. W., and Plotnick, L. (2008) Type 1 Diabetes Mellitus In Pediatrics. *Pediatrics In Review / American Academy of Pediatrics* **29**, 374-384; Quiz 385
5. Centers For Disease Control, (2010) Mortality Multiple Cause Micro-Data Files, U.S. Government DHHS, Atlanta, GA, U.S.A.
6. Van Dieren, S., Beulens, J. W., Van Der Schouw, Y. T., Grobbee, D. E., and Neal, B. (2010) The Global Burden of Diabetes and Its Complications: An Emerging Pandemic. *European Journal of Cardiovascular Prevention and Rehabilitation: Official Journal of the European Society of Cardiology, Working Groups On Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology* **17 Suppl 1**, S3-8
7. Farmer, L. (1952) Notes On The History of Diabetes Mellitus; Views Concerning Its Nature and Etiology Up to the Discovery of the Role of the Pancreas. *Bulletin of the New York Academy of Medicine* **28**, 408-416
8. Ahmed, A. M. (2002) History of Diabetes Mellitus. *Saudi Medical Journal* **23**, 373-378
9. Morabia, A. (2006) Claude Bernard Was A 19th Century Proponent of Medicine Based On Evidence. *Journal of Clinical Epidemiology* **59**, 1150-1154
10. John Rollo, M. D. (1797) An Account of Two Cases of the Diabetes Mellitus: With Remarks, As They Arose During The Progress of the Cure. To Which Are Added, A General View of the Nature of the Disease and Its Appropriate Treatment..., *In Two Volumes*, T. Gillet, London
11. Macleod, J. J. (1922) Insulin and Diabetes: A General Statement of the Physiological and Therapeutic Effects of Insulin. *British Medical Journal* **2**, 833-835
12. F.G. Banting, C. H. B. J. B. C. J. J. R. M. E. C. N. (1922) The Effect of Pancreatic Extract (Insulin) On Normal Rabbits. American Physiologial Society, Baltimore
13. Bliss, M. (1993) Rewriting Medical History: Charles Best and the Banting and Best Myth. *Journal of the History of Medicine and Allied Sciences* **48**, 253-274
14. Imperatore, G., Boyle, J. P., Thompson, T. J., Case, D., Dabelea, D., Hamman, R. F., Lawrence, J. M., Liese, A. D., Liu, L. L., Mayer-Davis, E. J., Rodriguez, B. L., Standiford, D., and Group, F. T. S. F. D. I. Y. S. (2012) Projections of Type 1 and Type 2 Diabetes Burden In The U.S. Population Aged <20 Years Through 2050: Dynamic Modeling of Incidence, Mortality, and Population Growth. *Diabetes Care* **35**, 2515-2520
15. Coustan, D. R. (2013) Gestational Diabetes Mellitus. *Clinical Chemistry* **59**, 1310-1321
16. Aguilar-Bryan, L., and Bryan, J. (2008) Neonatal Diabetes Mellitus. *Endocrine Reviews* **29**, 265-291
17. Shields, B. M., Hicks, S., Shepherd, M. H., Colclough, K., Hattersley, A. T., and Ellard, S. (2010) Maturity-Onset Diabetes of the Young (MODY): How Many Cases Are We Missing? *Diabetologia* **53**, 2504-2508

18. Association, A. D. (2008) Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* **31**, S55-S60
19. Awai, M., Narasaki, M., Yamanoi, Y., and Seno, S. (1979) Induction of Diabetes In Animals by Parenteral Administration of Ferric Nitrilotriacetate. A Model of Experimental Hemochromatosis. *The American Journal of Pathology* **95**, 663
20. Chauhan, H. K. (2012) Diabesity: The 'Achilles Heel' of Our Modernized Society. *Rev Assoc Med Bras* **58**, 399
21. Rhodes, C. J. (2005) Type 2 Diabetes-A Matter of Beta-Cell Life and Death? *Science* **307**, 380-384
22. Tamborlane, W. V., Beck, R. W., Bode, B. W., Buckingham, B., Chase, H. P., Clemons, R., Fiallo-Scharer, R., Fox, L. A., Gilliam, L. K., Hirsch, I. B., Huang, E. S., Kollman, C., Kowalski, A. J., Laffel, L., Lawrence, J. M., Lee, J., Mauras, N., O'grady, M., Ruedy, K. J., Tansey, M., Tsalikian, E., Weinzimer, S., Wilson, D. M., Wolpert, H., Wysocki, T., and Xing, D. (2008) Continuous Glucose Monitoring and Intensive Treatment of Type 1 Diabetes. *The New England Journal of Medicine* **359**, 1464-1476
23. Smyth, S., and Heron, A. (2006) Diabetes and Obesity: The Twin Epidemics. *Nature Medicine* **12**, 75-80
24. Konner, A. C., and Bruning, J. C. (2012) Selective Insulin and Leptin Resistance In Metabolic Disorders. *Cell Metabolism* **16**, 144-152
25. Rydén, L., Standl, E., Bartnik, M., Van Den Berghe, G., Betteridge, J., De Boer, M.-J., Cosentino, F., Jönsson, B., Laakso, M., Malmberg, K., Priori, S., Östergren, J., Tuomilehto, J., Thrainsdottir, I., Vanhorebeek, I., Stramba-Badiale, M., Lindgren, P., Qiao, Q., Priori, S. G., Blanc, J.-J., Budaj, A., Camm, J., Dean, V., Deckers, J., Dickstein, K., Lekakis, J., Mcgregor, K., Metra, M., Morais, J., Osterspey, A., Tamargo, J., Zamorano, J. L., Deckers, J. W., Bertrand, M., Charbonnel, B., Erdmann, E., Ferrannini, E., Flyvbjerg, A., Gohlke, H., Juanatey, J. R. G., Graham, I., Monteiro, P. F., Parhofer, K., Pyörälä, K., Raz, I., Schernthaner, G., Volpe, M., and Wood, D. (2007) Guidelines On Diabetes, Pre-Diabetes, and Cardiovascular Diseases: Executive Summary: The Task Force On Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association For The Study of Diabetes (EASD). *European Heart Journal* **28**, 88-136
26. Haffner, S. M. (2003) Pre-Diabetes, Insulin Resistance, Inflammation and CVD Risk. *Diabetes Research and Clinical Practice* **61, Supplement 1**, S9-S18
27. Fourlanos, S., Narendran, P., Byrnes, G. B., Colman, P. G., and Harrison, L. C. (2004) Insulin Resistance Is a Risk Factor for Progression to Type 1 Diabetes. *Diabetologia* **47**, 1661-1667
28. Schauer, I. E., Snell-Bergeon, J. K., Bergman, B. C., Maahs, D. M., Kretowski, A., Eckel, R. H., and Rewers, M. (2011) Insulin Resistance, Defective Insulin-Mediated Fatty Acid Suppression, and Coronary Artery Calcification In Subjects With and Without Type 1 Diabetes: The CACTI Study. *Diabetes* **60**, 306-314
29. Olefsky, J. M., and Glass, C. K. (2010) Macrophages, Inflammation, and Insulin Resistance. *Annual Review of Physiology* **72**, 219-246
30. Reaven, G. M. (1988) Role of Insulin Resistance In Human Disease. *Diabetes* **37**, 1595-1607
31. Lumeng, C. N., and Saltiel, A. R. (2011) Inflammatory Links Between Obesity and Metabolic Disease. *The Journal of Clinical Investigation* **121**, 2111

32. Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, M. L., Kagechika, H., Shudo, K., Yoda, M., Nakano, Y., Tobe, K., Nagai, R., Kimura, S., Tomita, M., Froguel, P., and Kadowaki, T. (2001) The Fat-Derived Hormone Adiponectin Reverses Insulin Resistance Associated With Both Lipoatrophy and Obesity. *Nature Medicine* **7**, 941-946
33. Corkey, B. E. (2012) Banting Lecture 2011: Hyperinsulinemia: Cause Or Consequence? *Diabetes* **61**, 4-13
34. Emanuelli, B., Peraldi, P., Filloux, C., Chavey, C., Freidinger, K., Hilton, D. J., Hotamisligil, G. S., and Van Obberghen, E. (2001) Socs-3 Inhibits Insulin Signaling and Is Up-Regulated In Response To Tumor Necrosis Factor-A In The Adipose Tissue of Obese Mice. *Journal of Biological Chemistry* **276**, 47944-47949
35. Hotamisligil, G., Shargill, N., and Spiegelman, B. (1993) Adipose Expression of Tumor Necrosis Factor-Alpha: Direct Role In Obesity-Linked Insulin Resistance. *Science* **259**, 87-91
36. Samuel, V. T., Petersen, K. F., and Shulman, G. I. (2010) Lipid-Induced Insulin Resistance: Unravelling the Mechanism. *The Lancet* **375**, 2267-2277
37. Oakes, N. D., Cooney, G. J., Camilleri, S., Chisholm, D. J., and Kraegen, E. W. (1997) Mechanisms of Liver and Muscle Insulin Resistance Induced by Chronic High-Fat Feeding. *Diabetes* **46**, 1768-1774
38. Kraegen, E. W., Clark, P. W., Jenkins, A. B., Daley, E. A., Chisholm, D. J., and Storlien, L. H. (1991) Development of Muscle Insulin Resistance After Liver Insulin Resistance In High-Fat–Fed Rats. *Diabetes* **40**, 1397-1403
39. (2013) Standards of Medical Care In Diabetes--2013. *Diabetes Care* **36 Suppl 1**, S11-66
40. Brod, M., Skovlund, S., and Wittrup-Jensen, K. (2006) Measuring the Impact of Diabetes Through Patient Report of Treatment Satisfaction, Productivity and Symptom Experience. *Quality of Life Research* **15**, 481-491
41. Association, A. D. (2011) Standards of Medical Care In Diabetes—2011. *Diabetes Care* **34**, S11
42. Rohlfing, C. L., Wiedmeyer, H.-M., Little, R. R., England, J. D., Tennill, A., and Goldstein, D. E. (2002) Defining the Relationship Between Plasma Glucose and Hba1c: Analysis of Glucose Profiles and Hba1c In The Diabetes Control and Complications Trial. *Diabetes Care* **25**, 275-278
43. Qaseem, A., Vijan, S., Snow, V., Cross, J. T., Weiss, K. B., and Owens, D. K. (2007) Glycemic Control and Type 2 Diabetes Mellitus: The Optimal Hemoglobin A1c Targets. A Guidance Statement From The American College of Physicians. *Annals of Internal Medicine* **147**, 417-422
44. Jeppsson, J. O., Kobold, U., Barr, J., Finke, A., Hoelzel, W., Hoshino, T., Miedema, K., Mosca, A., Mauri, P., Paroni, R., Thienpont, L., Umemoto, M., and Weykamp, C. (2002) Approved IFCC Reference Method for the Measurement of Hba1c In Human Blood. *Clinical Chemistry and Laboratory Medicine : Cclm / Fescc* **40**, 78-89
45. Bennett, C. M., Guo, M., and Dharmage, S. C. (2007) Hba1c as a Screening Tool For Detection of Type 2 Diabetes: A Systematic Review. *Diabetic Medicine* **24**, 333-343

46. Turner, R., Holman, R., Cull, C., Stratton, I., Matthews, D., and Frighi, V. (1998) Intensive Blood-Glucose Control With Sulphonylureas Or Insulin Compared With Conventional Treatment and Risk of Complications In Patients With Type 2 Diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* **352**, 837-853
47. Liddy, C., Dusseault, J. J., Dahrouge, S., Hogg, W., Lemelin, J., and Humbert, J. (2008) Telehomecare for Patients With Multiple Chronic Illnesses: Pilot Study. *Canadian Family Physician Medecin De Famille Canadien* **54**, 58-65
48. Selam, J. L., Slingeneyer, A., Chaptal, P. A., Franetzki, M., Prestele, K., and Mirouze, J. (1982) Total Implantation of a Remotely Controlled Insulin Minipump In a Human Insulin-Dependent Diabetic. *Artificial Organs* **6**, 315-319
49. Dungel, P., Long, N., Yu, B., Moussy, Y., and Moussy, F. (2008) Study of the Effects of Tissue Reactions On the Function of Implanted Glucose Sensors. *Journal of Biomedical Materials Research. Part A* **85**, 699-706
50. Steil, G. M., Rebrin, K., Darwin, C., Hariri, F., and Saad, M. F. (2006) Feasibility of Automating Insulin Delivery For the Treatment of Type 1 Diabetes. *Diabetes* **55**, 3344-3350
51. Haidar, A., Legault, L., Dallaire, M., Alkhateeb, A., Coriati, A., Messier, V., Cheng, P., Millette, M., Boulet, B., Huang, C. C., and Rabasa-Lhoret, R. (2013) Glucose-Responsive Insulin and Glucagon Delivery (Dual-Hormone Artificial Pancreas) In Adults With Type 1 Diabetes: A Randomized Crossover Controlled Trial. *CMAJ : Canadian Medical Association Journal = Journal De L'association Medicale Canadienne* **185**, 297-305
52. Egan, B., and Zierath, J. R. (2013) Exercise Metabolism and the Molecular Regulation of Skeletal Muscle Adaptation. *Cell Metabolism* **17**, 162-184
53. Inzucchi, S. E., Bergenstal, R. M., Buse, J. B., Diamant, M., Ferrannini, E., Nauck, M., Peters, A. L., Tsapas, A., Wender, R., and Matthews, D. R. (2012) Management of Hyperglycaemia In Type 2 Diabetes: A Patient-Centered Approach. Position Statement of the American Diabetes Association (ADA) and The European Association For The Study of Diabetes (EASD). *Diabetologia* **55**, 1577-1596
54. Tuomilehto, J., Lindstrom, J., Eriksson, J. G., Valle, T. T., Hamalainen, H., Ilanne-Parikka, P., Keinanen-Kiukaanniemi, S., Laakso, M., Louheranta, A., Rastas, M., Salminen, V., and Uusitupa, M. (2001) Prevention of Type 2 Diabetes Mellitus by Changes In Lifestyle Among Subjects With Impaired Glucose Tolerance. *The New England Journal of Medicine* **344**, 1343-1350
55. Bosi, E. (2009) Metformin--The Gold Standard In Type 2 Diabetes: What Does the Evidence Tell Us? *Diabetes, Obesity & Metabolism* **11 Suppl 2**, 3-8
56. Charbonnel, B., Penfornis, A., Varroud-Vial, M., Kusnik-Joinville, O., and Detournay, B. (2012) Insulin Therapy for Diabetes Mellitus: Treatment Regimens and Associated Costs. *Diabetes & Metabolism* **38**, 156-163
57. Currie, C. J., and Johnson, J. A. (2012) The Safety Profile of Exogenous Insulin In People With Type 2 Diabetes: Justification For Concern. *Diabetes, Obesity & Metabolism* **14**, 1-4
58. Nolan, C. J., Ruderman, N. B., and Prentki, M. (2013) Intensive Insulin for Type 2 Diabetes: The Risk of Causing Harm. *The Lancet Diabetes & Endocrinology* **1**, 9-10
59. Gomori, G. (1939) Studies On The Cells of the Pancreatic Islets. *The Anatomical Record* **74**
60. Busnardo, A. C., Didio, L. J., Tidrick, R. T., and Thomford, N. R. (1983) History of the Pancreas. *American Journal of Surgery* **146**, 539-550

61. Albert Von, K. (1863) *Handbuch Der Gewebelehre Des Menschen Für Aerzte U. Studirende*, W. Engelman
62. Delcroix, M., Sajid, M., Caffrey, C. R., Lim, K. C., Dvorak, J., Hsieh, I., Bahgat, M., Dissous, C., and Mckerrow, J. H. (2006) A Multienzyme Network Functions In Intestinal Protein Digestion by a Platyhelminth Parasite. *The Journal of Biological Chemistry* **281**, 39316-39329
63. Pieler, T., and Chen, Y. (2006) Forgotten and Novel Aspects In Pancreas Development. *Biology of the Cell / Under the Auspices of the European Cell Biology Organization* **98**, 79-88
64. Bunnag, S. C., Bunnag, S., and Warner, N. E. (1963) Microcirculation In the Islets of Langerhans of the Mouse. *The Anatomical Record* **146**, 117-123
65. Chen, N., Unnikrishnan, I. R., Anjana, R. M., Mohan, V., and Pitchumoni, C. S. (2011) The Complex Exocrine-Endocrine Relationship and Secondary Diabetes In Exocrine Pancreatic Disorders. *Journal of Clinical Gastroenterology* **45**, 850-861
66. Brissova, M., Shostak, A., Shiota, M., Wiebe, P. O., Poffenberger, G., Kantz, J., Chen, Z., Carr, C., Jerome, W. G., Chen, J., Baldwin, H. S., Nicholson, W., Bader, D. M., Jetton, T., Gannon, M., and Powers, A. C. (2006) Pancreatic Islet Production of Vascular Endothelial Growth Factor-A Is Essential For Islet Vascularization, Revascularization, and Function. *Diabetes* **55**, 2974-2985
67. Elayat, A. A., El-Naggar, M. M., and Tahir, M. (1995) An Immunocytochemical and Morphometric Study of the Rat Pancreatic Islets. *Journal of Anatomy* **186 (Pt 3)**, 629-637
68. Lacy, P. E. (1957) Electron Microscopic Identification of Different Cell Types In the Islets of Langerhans of the Guinea Pig, Rat, Rabbit and Dog. *The Anatomical Record* **128**, 255-267
69. Kelly, C., Mcclenaghan, N. H., and Flatt, P. R. (2011) Role of Islet Structure and Cellular Interactions In the Control of Insulin Secretion. *Islets* **3**, 41-47
70. Kim, A., Miller, K., Jo, J., Kilimnik, G., Wojcik, P., and Hara, M. (2009) Islet Architecture: A Comparative Study. *Islets* **1**, 129
71. Slack, J. M. (1995) Developmental Biology of the Pancreas. *Development* **121**, 1569-1580
72. Wilson, M. E., Scheel, D., and German, M. S. (2003) Gene Expression Cascades In Pancreatic Development. *Mechanisms of Development* **120**, 65-80
73. Sander, M., Neubüser, A., Kalamaras, J., Ee, H., Martin, G., and German, M. (1997) Genetic Analysis Reveals That Pax6 Is Required for Normal Transcription of Pancreatic Hormone Genes and Islet Development. *Genes & Development* **11**, 1662-1673
74. Dilorio, P., Moss, J., Sbrogna, J., Karlstrom, R., and Moss, L. (2002) Sonic Hedgehog Is Required Early In Pancreatic Islet Development. *Developmental Biology* **244**, 75-84
75. Murtaugh, L. C. (2007) Pancreas and Beta-Cell Development: From the Actual to the Possible. *Development* **134**, 427-438
76. Weir, G. C., and Bonner-Weir, S. (2004) Five Stages of Evolving Beta-Cell Dysfunction During Progression to Diabetes. *Diabetes* **53**, S16-S21
77. Talchai, C., Xuan, S., Lin, H. V., Sussel, L., and Accili, D. (2012) Pancreatic β Cell Dedifferentiation as a Mechanism of Diabetic β Cell Failure. *Cell* **150**, 1223-1234
78. Teta, M., Long, S. Y., Wartschow, L. M., Rankin, M. M., and Kushner, J. A. (2005) Very Slow Turnover of B-Cells In Aged Adult Mice. *Diabetes* **54**, 2557-2567

79. Høiriis Nielsen, J., Svensson, C., Douglas Galsgaard, E., Møldrup, A., and Billestrup, N. (1999) Beta Cell Proliferation and Growth Factors. *Journal of Molecular Medicine* **77**, 62-66
80. Bonner-Weir, S. (2000) Life and Death of the Pancreatic β Cells. *Trends In Endocrinology & Metabolism* **11**, 375-378
81. Sreenan, S., Pick, A. J., Levisetti, M., Baldwin, A. C., Pugh, W., and Polonsky, K. S. (1999) Increased Beta-Cell Proliferation and Reduced Mass Before Diabetes Onset In the Nonobese Diabetic Mouse. *Diabetes* **48**, 989-996
82. Xu, G., Stoffers, D. A., Habener, J. F., and Bonner-Weir, S. (1999) Exendin-4 Stimulates Both Beta-Cell Replication and Neogenesis, Resulting In Increased Beta-Cell Mass and Improved Glucose Tolerance In Diabetic Rats. *Diabetes* **48**, 2270-2276
83. Brubaker, P. L., and Drucker, D. J. (2004) Minireview: Glucagon-Like Peptides Regulate Cell Proliferation and Apoptosis In the Pancreas, Gut, and Central Nervous System. *Endocrinology* **145**, 2653-2659
84. Nielsen, J. H., Svensson, C., Galsgaard, E. D., Møldrup, A., and Billestrup, N. (1999) Beta Cell Proliferation and Growth Factors. *Journal of Molecular Medicine* **77**, 62-66
85. Kassem, S., Ariel, I., Thornton, P., Scheimberg, I., and Glaser, B. (2000) Beta-Cell Proliferation and Apoptosis In The Developing Normal Human Pancreas and In Hyperinsulinism of Infancy. *Diabetes* **49**, 1325-1333
86. Hao, E., Tyrberg, B., Itkin-Ansari, P., Lakey, J. R., Geron, I., Monosov, E. Z., Barcova, M., Mercola, M., and Levine, F. (2006) Beta-Cell Differentiation From Nonendocrine Epithelial Cells of the Adult Human Pancreas. *Nature Medicine* **12**, 310-316
87. Vasavada, R. C., Gonzalez-Pertusa, J. A., Fujinaka, Y., Fiaschi-Taesch, N., Cozar-Castellano, I., and Garcia-Ocaña, A. (2006) Growth Factors and Beta Cell Replication. *The International Journal of Biochemistry & Cell Biology* **38**, 931-950
88. Bonner-Weir, S. (2000) Islet Growth and Development In The Adult. *Journal of Molecular Endocrinology* **24**, 297-302
89. El Ouaamari, A., Kawamori, D., Dirice, E., Liew, Chong W., Shadrach, Jennifer L., Hu, J., Katsuta, H., Hollister-Lock, J., Qian, W.-J., Wagers, Amy J., and Kulkarni, Rohit N. (2013) Liver-Derived Systemic Factors Drive β Cell Hyperplasia In Insulin-Resistant States. *Cell Reports* **3**, 401-410
90. Hakonen, E., Ustinov, J., Mathijs, I., Palgi, J., Bouwens, L., Miettinen, P. J., and Otonkoski, T. (2011) Epidermal Growth Factor (EGF)-Receptor Signalling Is Needed for Murine Beta Cell Mass Expansion In Response to High-Fat Diet and Pregnancy But Not After Pancreatic Duct Ligation. *Diabetologia* **54**, 1735-1743
91. Bonner-Weir, S. (2001) Beta-Cell Turnover: Its Assessment and Implications. *Diabetes* **50**, S20
92. Ogilvie, R. F. (1933) The Islands of Langerhans In 19 Cases of Obesity. *The Journal of Pathology and Bacteriology* **37**, 473-481
93. Weir, G. C., Laybutt, D. R., Kaneto, H., Bonner-Weir, S., and Sharma, A. (2001) Beta-Cell Adaptation and Decompensation During the Progression of Diabetes. *Diabetes* **50 Suppl 1**, S154-159
94. Saisho, Y., Butler, A. E., Manesso, E., Elashoff, D., Rizza, R. A., and Butler, P. C. (2013) B-Cell Mass and Turnover In Humans: Effects of Obesity and Aging. *Diabetes Care* **36**, 111-117
95. Van Assche, F. A., Aerts, L., and De Prins, F. (1978) A Morphological Study of the Endocrine Pancreas In Human Pregnancy. *British Journal of Obstetrics and Gynaecology* **85**, 818-820

96. Rieck, S., and Kaestner, K. H. (2010) Expansion of B-Cell Mass In Response To Pregnancy. *Trends In Endocrinology & Metabolism* **21**, 151-158
97. Butler, A. E., Cao-Minh, L., Galasso, R., Rizza, R. A., Corradin, A., Cobelli, C., and Butler, P. C. (2010) Adaptive Changes In Pancreatic Beta Cell Fractional Area and Beta Cell Turnover In Human Pregnancy. *Diabetologia* **53**, 2167-2176
98. Jacovetti, C., Abderrahmani, A., Parnaud, G., Jonas, J. C., Peyot, M. L., Cornu, M., Laybutt, R., Meugnier, E., Rome, S., Thorens, B., Prentki, M., Bosco, D., and Regazzi, R. (2012) Micrornas Contribute to Compensatory Beta Cell Expansion During Pregnancy and Obesity. *The Journal of Clinical Investigation* **122**, 3541-3551
99. Wu, Y., Liu, C., Sun, H., Vijayakumar, A., Giglou, P. R., Qiao, R., Oppenheimer, J., Yakar, S., and Leroith, D. (2011) Growth Hormone Receptor Regulates Beta Cell Hyperplasia and Glucose-Stimulated Insulin Secretion In Obese Mice. *The Journal of Clinical Investigation* **121**, 2422-2426
100. Laybutt, D. R., Kaneto, H., Hasenkamp, W., Grey, S., Jonas, J. C., Sgroi, D. C., Groff, A., Ferran, C., Bonner-Weir, S., Sharma, A., and Weir, G. C. (2002) Increased Expression of Antioxidant and Antiapoptotic Genes In Islets That May Contribute to Beta-Cell Survival During Chronic Hyperglycemia. *Diabetes* **51**, 413-423
101. Chan, C. B., Macphail, R. M., Sheu, L., Wheeler, M. B., and Gaisano, H. Y. (1999) Beta-Cell Hypertrophy In Fa/Fa Rats Is Associated With Basal Glucose Hypersensitivity and Reduced Snare Protein Expression. *Diabetes* **48**, 997-1005
102. Donath, M. Y., Gross, D. J., Cerasi, E., and Kaiser, N. (1999) Hyperglycemia-Induced Beta-Cell Apoptosis In Pancreatic Islets of Psammomys Obesus During Development of Diabetes. *Diabetes* **48**, 738-744
103. Jewell, J. L., Oh, E., and Thurmond, D. C. (2010) Exocytosis Mechanisms Underlying Insulin Release and Glucose Uptake: Conserved Roles for Munc18c and Syntaxin 4. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **298**, R517-531
104. Guillam, M. T., Hummler, E., Schaerer, E., Yeh, J. I., Birnbaum, M. J., Beermann, F., Schmidt, A., Deriaz, N., and Thorens, B. (1997) Early Diabetes and Abnormal Postnatal Pancreatic Islet Development In Mice Lacking Glut-2. *Nature Genetics* **17**, 327-330
105. De Vos, A., Heimberg, H., Quartier, E., Huypens, P., Bouwens, L., Pipeleers, D., and Schuit, F. (1995) Human and Rat Beta Cells Differ In Glucose Transporter But Not In Glucokinase Gene Expression. *The Journal of Clinical Investigation* **96**, 2489-2495
106. Iynedjian, P. B. (2009) Molecular Physiology of Mammalian Glucokinase. *Cellular and Molecular Life Sciences : Cmls* **66**, 27-42
107. Krebs, H. A. (1970) The History of the Tricarboxylic Acid Cycle. *Perspectives In Biology and Medicine* **14**, 154-170
108. Tarasov, A., Dusonchet, J., and Ashcroft, F. (2004) Metabolic Regulation of the Pancreatic Beta-Cell ATP-Sensitive K+ Channel: A Pas De Deux. *Diabetes* **53 Suppl 3**, S113-122
109. German, M. S., Moss, L. G., and Rutter, W. J. (1990) Regulation of Insulin Gene Expression by Glucose and Calcium In Transfected Primary Islet Cultures. *The Journal of Biological Chemistry* **265**, 22063-22066
110. Ashcroft, F. M., Harrison, D. E., and Ashcroft, S. J. (1984) Glucose Induces Closure of Single Potassium Channels In Isolated Rat Pancreatic β-Cells. *Nature* **312**, 446-448

111. Wetherton, A. R., Corey, T. S., Buchino, J. J., and Burrows, A. M. (2003) Fatal Intravenous Injection of Potassium In Hospitalized Patients. *The American Journal of Forensic Medicine and Pathology* **24**, 128-131
112. Itoh, Y., Kawamata, Y., Harada, M., Kobayashi, M., Fujii, R., Fukusumi, S., Ogi, K., Hosoya, M., Tanaka, Y., Uejima, H., Tanaka, H., Maruyama, M., Satoh, R., Okubo, S., Kizawa, H., Komatsu, H., Matsumura, F., Noguchi, Y., Shinohara, T., Hinuma, S., Fujisawa, Y., and Fujino, M. (2003) Free Fatty Acids Regulate Insulin Secretion from Pancreatic Beta Cells Through GPR40. *Nature* **422**, 173-176
113. Bollheimer, L. C., Skelly, R. H., Chester, M. W., Mcgarry, J. D., and Rhodes, C. J. (1998) Chronic Exposure to Free Fatty Acid Reduces Pancreatic Beta Cell Insulin Content by Increasing Basal Insulin Secretion That Is Not Compensated For by a Corresponding Increase In Proinsulin Biosynthesis Translation. *The Journal of Clinical Investigation* **101**, 1094-1101
114. Bohannon, N. V., Karam, J. H., and Forsham, P. H. (1980) Endocrine Responses to Sugar Ingestion In Man. Advantages of Fructose Over Sucrose and Glucose. *Journal of the American Dietetic Association* **76**, 555-560
115. Zavaroni, I., Sander, S., Scott, S., and Reaven, G. M. (1980) Effect of Fructose Feeding On Insulin Secretion and Insulin Action In the Rat. *Metabolism: Clinical and Experimental* **29**, 970-973
116. Aguilar-Bryan, L., Nichols, C., Wechsler, S., Clement, J., Boyd, A., Gonzalez, G., Herrera-Sosa, H., Nguy, K., Bryan, J., and Nelson, D. (1995) Cloning of the Beta Cell High-Affinity Sulfonylurea Receptor: A Regulator of Insulin Secretion. *Science* **268**, 423-426
117. Shin, M. S., Yu, J. H., Jung, C. H., Hwang, J. Y., Lee, W. J., Kim, M. S., and Park, J. Y. (2012) The Duration of Sulfonylurea Treatment Is Associated With Beta-Cell Dysfunction In Patients With Type 2 Diabetes Mellitus. *Diabetes Technology & Therapeutics* **14**, 1033-1042
118. Leroith, D. (2002) Beta-Cell Dysfunction and Insulin Resistance In Type 2 Diabetes: Role of Metabolic and Genetic Abnormalities. *The American Journal of Medicine* **113 Suppl 6a**, 3s-11s
119. Nielsen, J. H., Galsgaard, E. D., Møldrup, A., Friedrichsen, B. N., Billestrup, N., Hansen, J. A., Lee, Y. C., and Carlsson, C. (2001) Regulation of Beta-Cell Mass by Hormones and Growth Factors. *Diabetes* **50**, S25
120. Kjems, L. L., Holst, J. J., Vølund, A., and Madsbad, S. (2003) The Influence of Glp-1 On Glucose-Stimulated Insulin Secretion: Effects On β-Cell Sensitivity In Type 2 and Nondiabetic Subjects. *Diabetes* **52**, 380-386
121. Ahren, B., and Schmitz, O. (2004) GLP-1 Receptor Agonists and DPP-4 Inhibitors In the Treatment of Type 2 Diabetes. *Hormone and Metabolic Research = Hormon- und Stoffwechselforschung = Hormones et Metabolisme* **36**, 867-876
122. Rehfeld, J. F., and Stadil, F. (1973) The Effect of Gastrin On Basal- and Glucose-Stimulated Insulin Secretion In Man. *Journal of Clinical Investigation* **52**, 1415
123. Rehfeld, J. F. (2011) Incretin Physiology Beyond Glucagon-Like Peptide 1 and Glucose-Dependent Insulinotropic Polypeptide: Cholecystokinin and Gastrin Peptides. *Acta Physiologica* **201**, 405-411
124. Rodriguez-Diaz, R., Dando, R., Jacques-Silva, M. C., Fachado, A., Molina, J., Abdulreda, M. H., Ricordi, C., Roper, S. D., Berggren, P. O., and Caicedo, A. (2011) Alpha Cells Secrete Acetylcholine as a Non-Neuronal Paracrine Signal Priming Beta Cell Function In Humans. *Nature Medicine* **17**, 888-892

125. Razavi, R., Chan, Y., Afifiyan, F. N., Liu, X. J., Wan, X., Yantha, J., Tsui, H., Tang, L., Tsai, S., Santamaria, P., Driver, J. P., Serreze, D., Salter, M. W., and Dosch, H. M. (2006) TRPV1+ Sensory Neurons Control β Cell Stress and Islet Inflammation In Autoimmune Diabetes. *Cell* **127**, 1123-1135
126. Suri, A., and Szallasi, A. (2008) The Emerging Role of TRPV1 In Diabetes and Obesity. *Trends In Pharmacological Sciences* **29**, 29-36
127. Steiner, D. F., Park, S. Y., Stoy, J., Philipson, L. H., and Bell, G. I. (2009) A Brief Perspective On Insulin Production. *Diabetes, Obesity & Metabolism* **11 Suppl 4**, 189-196
128. Rao, R. V., Hermel, E., Castro-Obregon, S., Del Rio, G., Ellerby, L. M., Ellerby, H. M., and Bredesen, D. E. (2001) Coupling Endoplasmic Reticulum Stress to the Cell Death Program. Mechanism of Caspase Activation. *The Journal of Biological Chemistry* **276**, 33869-33874
129. Jansen, G., Maattanen, P., Denisov, A. Y., Scarffe, L., Schade, B., Balghi, H., Dejgaard, K., Chen, L. Y., Muller, W. J., Gehring, K., and Thomas, D. Y. (2012) An Interaction Map of Endoplasmic Reticulum Chaperones and Foldases. *Molecular & Cellular Proteomics : Mcp* **11**, 710-723
130. Simon, S. M., and Blobel, G. (1991) A Protein-Conducting Channel In the Endoplasmic Reticulum. *Cell* **65**, 371-380
131. Laybutt, D. R., Preston, A. M., Åkerfeldt, M. C., Kench, J. G., Busch, A. K., Biankin, A. V., and Biden, T. J. (2007) Endoplasmic Reticulum Stress Contributes to Beta Cell Apoptosis In Type 2 Diabetes. *Diabetologia* **50**, 752-763
132. Prentki, M., and Nolan, C. J. (2006) Islet Beta Cell Failure In Type 2 Diabetes. *The Journal of Clinical Investigation* **116**, 1802-1812
133. Özcan, U., Cao, Q., Yilmaz, E., Lee, A.-H., Iwakoshi, N. N., Özdelen, E., Tuncman, G., Görgün, C., Glimcher, L. H., and Hotamisligil, G. S. (2004) Endoplasmic Reticulum Stress Links Obesity, Insulin Action, and Type 2 Diabetes. *Science* **306**, 457-461
134. Bánhegyi, G., Baumeister, P., Benedetti, A., Dong, D., Fu, Y., Lee, A. S., Li, J., Mao, C., Margittai, É., Ni, M. I. N., Paschen, W., Piccirella, S., Senesi, S., Sitia, R., Wang, M., and Yang, W. E. I. (2007) Endoplasmic Reticulum Stress. *Annals of the New York Academy of Sciences* **1113**, 58-71
135. Araki, E., Oyadomari, S., and Mori, M. (2003) Endoplasmic Reticulum Stress and Diabetes Mellitus. *Intern Med* **42**, 7-14
136. Eizirik, D. L., Cardozo, A. K., and Cnop, M. (2008) The Role for Endoplasmic Reticulum Stress In Diabetes Mellitus. *Endocrine Reviews* **29**, 42-61
137. Bedard, K., Szabo, E., Michalak, M., and Opas, M. (2005) Cellular Functions of Endoplasmic Reticulum Chaperones Calreticulin, Calnexin, and Erp57. *Int. Rev. Cytol.* **245**, 91-121
138. Lee, A. H., Iwakoshi, N. N., and Glimcher, L. H. (2003) Xbp-1 Regulates A Subset of Endoplasmic Reticulum Resident Chaperone Genes In the Unfolded Protein Response. *Molecular and Cellular Biology* **23**, 7448-7459
139. Ozawa, K., Miyazaki, M., Matsuhisa, M., Takano, K., Nakatani, Y., Hatazaki, M., Tamatani, T., Yamagata, K., Miyagawa, J.-I., Kitao, Y., Hori, O., Yamasaki, Y., and Ogawa, S. (2005) The Endoplasmic Reticulum Chaperone Improves Insulin Resistance In Type 2 Diabetes. *Diabetes* **54**, 657-663
140. Gorlach, A., Klappa, P., and Kietzmann, T. (2006) The Endoplasmic Reticulum: Folding, Calcium Homeostasis, Signaling, and Redox Control. *Antioxidants & Redox Signaling* **8**, 1391-1418

141. Kim, J. H., Johannes, L., Goud, B., Antony, C., Lingwood, C. A., Daneman, R., and Grinstein, S. (1998) Noninvasive Measurement of the pH of the Endoplasmic Reticulum at Rest and During Calcium Release. *Proceedings of the National Academy of Sciences* **95**, 2997-3002
142. Didomenico, B. J., Bugaisky, G. E., and Lindquist, S. (1982) The Heat Shock Response Is Self-Regulated at Both the Transcriptional and Posttranscriptional Levels. *Cell* **31**, 593-603
143. Corbett, E. F., Oikawa, K., Francois, P., Tessier, D. C., Kay, C., Bergeron, J. J. M., Thomas, D. Y., Krause, K.-H., and Michalak, M. (1999) Ca2+ Regulation of Interactions Between Endoplasmic Reticulum Chaperones. *Journal of Biological Chemistry* **274**, 6203-6211
144. Evans-Molina, C., Hatanaka, M., and Mirmira, R. G. (2013) Lost In Translation: Endoplasmic Reticulum Stress and the Decline of Beta-Cell Health In Diabetes Mellitus. *Diabetes, Obesity & Metabolism* **15 Suppl 3**, 159-169
145. Harding, H. P., and Ron, D. (2002) Endoplasmic Reticulum Stress and the Development of Diabetes: A Review. *Diabetes* **51 Suppl 3**, S455-461
146. Ramadan, J. W., Steiner, S. R., O'neill, C. M., and Nunemaker, C. S. (2011) The Central Role of Calcium In the Effects of Cytokines On Beta-Cell Function: Implications for Type 1 and Type 2 Diabetes. *Cell Calcium* **50**, 481-490
147. Liang, S.-H., Zhang, W., Mcgrath, B. C., Zhang, P., and Cavener, D. R. (2006) PERK (EIF2α Kinase) Is Required to Activate the Stress-Activated MAPKs and Induce the Expression of Immediate-Early Genes Upon Disruption of ER Calcium Homoeostasis. *The Biochemical Journal* **393**, 201-209
148. Chakrabarti, A., Chen, A. W., and Varner, J. D. (2011) A Review of the Mammalian Unfolded Protein Response. *Biotechnology and Bioengineering* **108**, 2777-2793
149. Sturgess, N., Cook, D., Ashford, M. J., and Hales, C. N. (1985) The Sulphonylurea Receptor May Be an ATP-Sensitive Potassium Channel. *The Lancet* **326**, 474-475
150. Duchen, M. R., Smith, P. A., and Ashcroft, F. M. (1993) Substrate-Dependent Changes In Mitochondrial Function, Intracellular Free Calcium Concentration and Membrane Channels In Pancreatic Beta-Cells. *The Biochemical Journal* **294 (Pt 1)**, 35-42
151. Muoio, D. M., and Newgard, C. B. (2008) Mechanisms of Disease: Molecular and Metabolic Mechanisms of Insulin Resistance and Beta-Cell Failure In Type 2 Diabetes. *Nature Reviews. Molecular Cell Biology* **9**, 193-205
152. Bertram, R., Smolen, P., Sherman, A., Mears, D., Atwater, I., Martin, F., and Soria, B. (1995) A Role for Calcium Release-Activated Current (CRAC) In Cholinergic Modulation of Electrical Activity In Pancreatic Beta-Cells. *Biophysical Journal* **68**, 2323-2332
153. Worley, J. F., Mcintyre, M. S., Spencer, B., Mertz, R. J., Roe, M. W., and Dukes, I. D. (1994) Endoplasmic Reticulum Calcium Store Regulates Membrane Potential In Mouse Islet Beta-Cells. *Journal of Biological Chemistry* **269**, 14359-14362
154. Wiederkehr, A., and Wollheim, C. B. (2008) Impact of Mitochondrial Calcium On The Coupling of Metabolism to Insulin Secretion In the Pancreatic β-Cell. *Cell Calcium* **44**, 64-76
155. Kono, T., Ahn, G., Moss, D. R., Gann, L., Zarain-Herzberg, A., Nishiki, Y., Fueger, P. T., Ogihara, T., and Evans-Molina, C. (2012) PPAR-Gamma Activation Restores Pancreatic Islet SERCA2 Levels and Prevents Beta-Cell

Dysfunction Under Conditions of Hyperglycemic and Cytokine Stress. *Mol Endocrinol* **26**, 257-271
156. Rutkowski, D. T., and Kaufman, R. J. (2007) That Which Does Not Kill Me Makes Me Stronger: Adapting to Chronic ER Stress. *Trends In Biochemical Sciences* **32**, 469-476
157. Michalak, M., Robert Parker, J. M., and Opas, M. (2002) Ca2+ Signaling and Calcium Binding Chaperones of the Endoplasmic Reticulum. *Cell Calcium* **32**, 269-278
158. Corbett, E. F., and Michalak, M. (2000) Calcium, A Signaling Molecule In the Endoplasmic Reticulum? *Trends In Biochemical Sciences* **25**, 307-311
159. Jansen, G., Maattanen, P., Denisov, A. Y., Scarffe, L., Schade, B., Balghi, H., Dejgaard, K., Chen, L. Y., Muller, W. J., Gehring, K., and Thomas, D. Y. (2012) An Interaction Map of ER Chaperones and Foldases. *Molecular & Cellular Proteomics : Mcp*
160. Pinton, P., Giorgi, C., Siviero, R., Zecchini, E., and Rizzuto, R. (2008) Calcium and Apoptosis: ER-Mitochondria Ca2+ Transfer In The Control of Apoptosis. *Oncogene* **27**, 6407-6418
161. Orrenius, S., Zhivotovsky, B., and Nicotera, P. (2003) Regulation of Cell Death: The Calcium-Apoptosis Link. *Nature Reviews. Molecular Cell Biology* **4**, 552-565
162. Wang, Y., Gao, L., Li, Y., Chen, H., and Sun, Z. (2011) Nifedipine Protects INS-1 Beta-Cell From High Glucose-Induced ER Stress and Apoptosis. *International Journal of Molecular Sciences* **12**, 7569-7580
163. Kharroubi, I., Ladrière, L., Cardozo, A. K., Dogusan, Z., Cnop, M., and Eizirik, D. L. (2004) Free Fatty Acids and Cytokines Induce Pancreatic β-Cell Apoptosis by Different Mechanisms: Role of Nuclear Factor-κB and Endoplasmic Reticulum Stress. *Endocrinology* **145**, 5087-5096
164. Csordás, G., and Hajnóczky, G. (2009) SR/ER–Mitochondrial Local Communication: Calcium and ROS. *Biochimica Et Biophysica Acta (BBA)-Bioenergetics* **1787**, 1352-1362
165. Zhang, K., and Kaufman, R. J. (2008) From Endoplasmic-Reticulum Stress to the Inflammatory Response. *Nature* **454**, 455-462
166. Schroder, M., and Kaufman, R. J. (2005) The Mammalian Unfolded Protein Response. *Annual Review of Biochemistry* **74**, 739-789
167. Olzmann, J. A., Kopito, R. R., and Christianson, J. C. (2013) The Mammalian Endoplasmic Reticulum-Associated Degradation System. *Cold Spring Harbor Perspectives In Biology* **5**
168. Harding, H. P., Zeng, H., Zhang, Y., Jungries, R., Chung, P., Plesken, H., Sabatini, D. D., and Ron, D. (2001) Diabetes Mellitus and Exocrine Pancreatic Dysfunction In PERK-/- Mice Reveals a Role for Translational Control In Secretory Cell Survival. *Molecular Cell* **7**, 1153-1163
169. Mckimpson, W. M., Weinberger, J., Czerski, L., Zheng, M., Crow, M. T., Pessin, J. E., Chua, S. C., Jr., and Kitsis, R. N. (2012) The Apoptosis Inhibitor ARC Alleviates The ER Stress Response to Promote Beta-Cell Survival. *Diabetes*
170. Sachdeva, M. M., Claiborn, K. C., Khoo, C., Yang, J., Groff, D. N., Mirmira, R. G., and Stoffers, D. A. (2009) Pdx1 (MODY4) Regulates Pancreatic Beta Cell Susceptibility To ER Stress. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 19090-19095

171. Shimizu, S., Hosooka, T., Matsuda, T., Asahara, S.-I., Koyanagi-Kimura, M., Kanno, A., Bartolome, A., Etoh, H., Fuchita, M., Teruyama, K., Takahashi, H., Inoue, H., Mieda, Y., Hashimoto, N., Seino, S., and Kido, Y. (2012) DPP4 Inhibitor Vildagliptin Preserves β-Cell Mass Through Amelioration of Endoplasmic Reticulum Stress In C/EBP Transgenic Mice. *Journal of Molecular Endocrinology* **49**, 125-135
172. Oslowski, Christine M., Hara, T., O'sullivan-Murphy, B., Kanekura, K., Lu, S., Hara, M., Ishigaki, S., Zhu, Lihua J., Hayashi, E., Hui, Simon T., Greiner, D., Kaufman, Randal J., Bortell, R., and Urano, F. (2012) Thioredoxin-Interacting Protein Mediates ER Stress-Induced β Cell Death Through Initiation of the Inflammasome. *Cell Metabolism* **16**, 265-273
173. Wu, J., and Kaufman, R. J. (2006) From Acute ER Stress To Physiological Roles of the Unfolded Protein Response. *Cell Death and Differentiation* **13**, 374-384
174. Vangheluwe, P., Raeymaekers, L., Dode, L., and Wuytack, F. (2005) Modulating Sarco(Endo)Plasmic Reticulum Ca2+ ATPase 2 (SERCA2) Activity: Cell Biological Implications. *Cell Calcium* **38**, 291-302
175. Grover, A. K., and Khan, I. (1992) Calcium Pump Isoforms: Diversity, Selectivity and Plasticity. Review Article. *Cell Calcium* **13**, 9-17
176. Inesi, G., Lewis, D., Ma, H., Prasad, A., and Toyoshima, C. (2006) Concerted Conformational Effects of Ca2+ and ATP Are Required for Activation of Sequential Reactions In The Ca2+ ATPase (SERCA) Catalytic Cycle. *Biochemistry* **45**, 13769-13778
177. Carafoli, E., and Brini, M. (2000) Calcium Pumps: Structural Basis for and Mechanism of Calcium Transmembrane Transport. *Current Opinion In Chemical Biology* **4**, 152-161
178. Mueller, B., Zhao, M., Negrashov, I. V., Bennett, R., and Thomas, D. D. (2004) SERCA Structural Dynamics Induced by ATP and Calcium. *Biochemistry* **43**, 12846-12854
179. Vafiadaki, E., Arvanitis, D. A., Pagakis, S. N., Papalouka, V., Sanoudou, D., Kontrogianni-Konstantopoulos, A., and Kranias, E. G. (2009) The Anti-Apoptotic Protein Hax-1 Interacts With SERCA2 and Regulates Its Protein Levels To Promote Cell Survival. *Molecular Biology of the Cell* **20**, 306-318
180. Ahlers, B. A., Song, J., Wang, J., Zhang, X. Q., Carl, L. L., Tadros, G. M., Rothblum, L. I., and Cheung, J. Y. (2005) Effects of Sarcoplasmic Reticulum Ca2+-ATPase Overexpression In Postinfarction Rat Myocytes. *J Appl Physiol (1985)* **98**, 2169-2176
181. Burke, B. E., Olson, R. D., Cusack, B. J., Gambliel, H. A., and Dillmann, W. H. (2003) Anthracycline Cardiotoxicity In Transgenic Mice Overexpressing SR Ca2+-ATPase. *Biochemical and Biophysical Research Communications* **303**, 504-507
182. Scorrano, L., Oakes, S. A., Opferman, J. T., Cheng, E. H., Sorcinelli, M. D., Pozzan, T., and Korsmeyer, S. J. (2003) Bax and Bak Regulation of Endoplasmic Reticulum Ca2+: A Control Point for Apoptosis. *Science* **300**, 135-139
183. He, H., Giordano, F. J., Hilal-Dandan, R., Choi, D. J., Rockman, H. A., Mcdonough, P. M., Bluhm, W. F., Meyer, M., Sayen, M. R., Swanson, E., and Dillmann, W. H. (1997) Overexpression of the Rat Sarcoplasmic Reticulum Ca2+ ATPase Gene In The Heart of Transgenic Mice Accelerates Calcium Transients and Cardiac Relaxation. *The Journal of Clinical Investigation* **100**, 380-389

184. Xu, G. G., Gao, Z. Y., Borge, P. D., Jr., Jegier, P. A., Young, R. A., and Wolf, B. A. (2000) Insulin Regulation of Beta-Cell Function Involves a Feedback Loop On SERCA Gene Expression, Ca(2+) Homeostasis, and Insulin Expression and Secretion. *Biochemistry* **39**, 14912-14919
185. Arredouani, A., Guiot, Y., Jonas, J. C., Liu, L. H., Nenquin, M., Pertusa, J. A., Rahier, J., Rolland, J. F., Shull, G. E., Stevens, M., Wuytack, F., Henquin, J. C., and Gilon, P. (2002) SERCA3 Ablation Does Not Impair Insulin Secretion but Suggests Distinct Roles of Different Sarcoendoplasmic Reticulum Ca(2+) Pumps for Ca(2+) Homeostasis In Pancreatic Beta-Cells. *Diabetes* **51**, 3245-3253
186. Varadi, A., and Rutter, G. A. (2002) Dynamic Imaging of Endoplasmic Reticulum Ca2+ Concentration In Insulin-Secreting MIN6 Cells Using Recombinant Targeted Cameleons: Roles of Sarco(Endo)Plasmic Reticulum Ca2+-ATPase (SERCA)-2 and Ryanodine Receptors. *Diabetes* **51 Suppl 1**, S190-201
187. Vetter, R., Rehfeld, U., Reissfelder, C., Weiss, W., Wagner, K.-D., Günther, J., Hammes, A., Tschöpe, C., Dillmann, W., and Paul, M. (2002) Transgenic Overexpression of the Sarcoplasmic Reticulum Ca2+ATPase Improves Reticular Ca2+ Handling In Normal and Diabetic Rat Hearts. *The Faseb Journal* **16**, 1657-1659
188. Zhao, X. S., Shin, D. M., Liu, L. H., Shull, G. E., and Muallem, S. (2001) Plasticity and Adaptation of Ca2+ Signaling and Ca2+-Dependent Exocytosis In SERCA2(+/-) Mice. *The Embo Journal* **20**, 2680-2689
189. Periasamy, M., Reed, T. D., Liu, L. H., Ji, Y., Loukianov, E., Paul, R. J., Nieman, M. L., Riddle, T., Duffy, J. J., Doetschman, T., Lorenz, J. N., and Shull, G. E. (1999) Impaired Cardiac Performance In Heterozygous Mice With a Null Mutation In the Sarco(Endo)Plasmic Reticulum Ca2+-ATPase Isoform 2 (SERCA2) Gene. *Journal of Biological Chemistry* **274**, 2556-2562
190. Ji, Y., Lalli, M. J., Babu, G. J., Xu, Y., Kirkpatrick, D. L., Liu, L. H., Chiamvimonvat, N., Walsh, R. A., Shull, G. E., and Periasamy, M. (2000) Disruption of a Single Copy of the SERCA2 Gene Results In Altered Ca2+ Homeostasis and Cardiomyocyte Function. *Journal of Biological Chemistry* **275**, 38073-38080
191. Sakuntabhai, A., Burge, S., Monk, S., and Hovnanian, A. (1999) Spectrum of Novel ATP2a2 Mutations In Patients With Darier's Disease. *Human Molecular Genetics* **8**, 1611-1619
192. Ferencz, C., Rubin, J. D., Mccarter, R. J., and Clark, E. B. (1990) Maternal Diabetes and Cardiovascular Malformations: Predominance of Double Outlet Right Ventricle and Truncus Arteriosus. *Teratology* **41**, 319-326
193. Prasad, V., Okunade, G. W., Miller, M. L., and Shull, G. E. (2004) Phenotypes of SERCA and PMCA Knockout Mice. *Biochemical and Biophysical Research Communications* **322**, 1192-1203
194. Verboomen, H., Wuytack, F., De Smedt, H., Himpens, B., and Casteels, R. (1992) Functional Difference Between SERCA2a and SERCA2b Ca2+ Pumps and Their Modulation by Phospholamban. *Biochem. J* **286**, 591-595
195. Vangheluwe, P., Raeymaekers, L., Dode, L., and Wuytack, F. (2005) Modulating Sarco(Endo)Plasmic Reticulum Ca2+ ATPase 2 (SERCA2) Activity: Cell Biological Implications. *Cell Calcium* **38**, 291-302
196. Shull, G. E. (2000) Gene Knockout Studies of Ca2+-Transporting ATPases. *European Journal of Biochemistry / Febs* **267**, 5284-5290
197. Zarain-Herzberg, A., and Alvarez-Fernandez, G. (2002) Sarco(Endo)Plasmic Reticulum Ca2+-ATPase-2 Gene: Structure and Transcriptional Regulation of the Human Gene. *Thescientificworldjournal* **2**, 1469-1483

198. Roderick, H. L., Lechleiter, J. D., and Camacho, P. (2000) Cytosolic Phosphorylation of Calnexin Controls Intracellular Ca(2+) Oscillations Via an Interaction With SERCA2b. *The Journal of Cell Biology* **149**, 1235-1248
199. Gorski, P. A., Trieber, C. A., Larivière, E., Schuermans, M., Wuytack, F., Young, H. S., and Vangheluwe, P. (2012) Transmembrane Helix 11 Is a Genuine Regulator of the Endoplasmic Reticulum Ca2+ Pump and Acts as a Functional Parallel of B-Subunit On A-Na+,K+-ATPase. *Journal of Biological Chemistry* **287**, 19876-19885
200. Clausen, J. D., Vandecaetsbeek, I., Wuytack, F., Vangheluwe, P., and Andersen, J. P. (2012) Distinct Roles of the C-Terminal 11th Transmembrane Helix and Luminal Extension In the Partial Reactions Determining the High Ca2+ Affinity of Sarco(Endo)Plasmic Reticulum Ca2+-ATPase Isoform 2b (SERCA2b). *Journal of Biological Chemistry* **287**, 39460-39469
201. Evans-Molina, C., Robbins, R. D., Kono, T., Tersey, S. A., Vestermark, G. L., Nunemaker, C. S., Garmey, J. C., Deering, T. G., Keller, S. R., Maier, B., and Mirmira, R. G. (2009) Peroxisome Proliferator-Activated Receptor Gamma Activation Restores Islet Function In Diabetic Mice Through Reduction of Endoplasmic Reticulum Stress and Maintenance of Euchromatin Structure. *Molecular and Cellular Biology* **29**, 2053-2067
202. Roe, M. W., Philipson, L. H., Frangakis, C. J., Kuznetsov, A., Mertz, R. J., Lancaster, M. E., Spencer, B., Worley, J. F., 3rd, and Dukes, I. D. (1994) Defective Glucose-Dependent Endoplasmic Reticulum Ca2+ Sequestration In Diabetic Mouse Islets of Langerhans. *The Journal of Biological Chemistry* **269**, 18279-18282
203. Cardozo, A. K., Ortis, F., Storling, J., Feng, Y. M., Rasschaert, J., Tonnesen, M., Van Eylen, F., Mandrup-Poulsen, T., Herchuelz, A., and Eizirik, D. L. (2005) Cytokines Downregulate the Sarcoendoplasmic Reticulum Pump Ca2+ ATPase 2b and Deplete Endoplasmic Reticulum Ca2+, Leading to Induction of Endoplasmic Reticulum Stress In Pancreatic Beta-Cells. *Diabetes* **54**, 452-461
204. Hu, P., Yin, C., Zhang, K. M., Wright, L. D., Nixon, T. E., Wechsler, A. S., Spratt, J. A., and Briggs, F. N. (1995) Transcriptional Regulation of Phospholamban Gene and Translational Regulation of SERCA2 Gene Produces Coordinate Expression of These Two Sarcoplasmic Reticulum Proteins During Skeletal Muscle Phenotype Switching. *The Journal of Biological Chemistry* **270**, 11619-11622
205. John, L. M., Lechleiter, J. D., and Camacho, P. (1998) Differential Modulation of SERCA2 Isoforms by Calreticulin. *The Journal of Cell Biology* **142**, 963-973
206. Li, Y., and Camacho, P. (2004) Ca2+-Dependent Redox Modulation of SERCA2b by Erp57. *The Journal of Cell Biology* **164**, 35-46
207. Thuerauf, D. J., Hoover, H., Meller, J., Hernandez, J., Su, L., Andrews, C., Dillmann, W. H., Mcdonough, P. M., and Glembotski, C. C. (2001) Sarco/Endoplasmic Reticulum Calcium ATPase-2 Expression Is Regulated by Atf6 During the Endoplasmic Reticulum Stress Response: Intracellular Signaling of Calcium Stress In a Cardiac Myocyte Model System. *Journal of Biological Chemistry* **276**, 48309-48317
208. Ihara, Y., Kageyama, K., and Kondo, T. (2005) Overexpression of Calreticulin Sensitizes SERCA2a to Oxidative Stress. *Biochemical and Biophysical Research Communications* **329**, 1343-1349
209. Hojmann Larsen, A., Frandsen, A., and Treiman, M. (2001) Upregulation of the SERCA-Type Ca2+ Pump Activity In Response to Endoplasmic Reticulum Stress In PC12 Cells. *BMC Biochemistry* **2**, 4

210. Koitabashi, N., Arai, M., Tomaru, K., Takizawa, T., Watanabe, A., Niwano, K., Yokoyama, T., Wuytack, F., Periasamy, M., Nagai, R., and Kurabayashi, M. (2005) Carvedilol Effectively Blocks Oxidative Stress-Mediated Downregulation of Sarcoplasmic Reticulum Ca2+-ATPase 2 Gene Transcription Through Modification of Sp1 Binding. *Biochemical and Biophysical Research Communications* **328**, 116-124
211. Caspersen, C., Pedersen, P. S., and Treiman, M. (2000) The Sarco-Endoplasmic Reticulum Calcium-ATPase 2b Is an Endoplasmic Reticulum Stress-Inducible Protein. *Journal of Biological Chemistry* **275** 22363-72
212. Wu, C.-K., Lee, J.-K., Chiang, F.-T., Yang, C.-H., Huang, S.-W., Hwang, J.-J., Lin, J.-L., Tseng, C.-D., Chen, J.-J., and Tsai, C.-T. (2011) Plasma Levels of Tumor Necrosis Factor-A and Interleukin-6 Are Associated With Diastolic Heart Failure Through Downregulation of Sarcoplasmic Reticulum Ca2+ ATPase. *Critical Care Medicine* **39**, 984-992 910
213. Villegas, S., Villarreal, F. J., and Dillmann, W. H. (2000) Leukemia Inhibitory Factor and Interleukin-6 Downregulate Sarcoplasmic Reticulum Ca2+ ATPase (SERCA2) In Cardiac Myocytes. *Basic Res Cardiol* **95**, 47-54
214. Seth, M., Sumbilla, C., Mullen, S. P., Lewis, D., Klein, M. G., Hussain, A., Soboloff, J., Gill, D. L., and Inesi, G. (2004) Sarco(Endo)Plasmic Reticulum Ca2+ ATPase (SERCA) Gene Silencing and Remodeling of the Ca2+ Signaling Mechanism In Cardiac Myocytes. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 16683-16688
215. Wu, K.-D., Bungard, D., and Lytton, J. (2001) Regulation of SERCA Ca2+ Pump Expression by Cytoplasmic [Ca2+] In Vascular Smooth Muscle Cells. *American Journal of Physiology-Cell Physiology* **280**, C843-C851
216. Rupp, H., and Zarain-Herzberg, A. (2002) Therapeutic Potential of CPT I Inhibitors: Cardiac Gene Transcription as a Target. *Expert Opinion On Investigational Drugs* **11**, 345-356
217. Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Wahli, W. (1996) Differential Expression of Peroxisome Proliferator-Activated Receptors (PPARs): Tissue Distribution of PPAR-Alpha, -Beta, and -Gamma In the Adult Rat. *Endocrinology* **137**, 354-366
218. Randriamboavonjy, V., Pistrosch, F., Bölck, B., Schwinger, R. H., Dixit, M., Badenhoop, K., Cohen, R. A., Busse, R., and Fleming, I. (2008) Platelet Sarcoplasmic Endoplasmic Reticulum Ca2+-ATPase and M-Calpain Activity Are Altered In Type 2 Diabetes Mellitus and Restored by Rosiglitazone. *Circulation* **117**, 52-60
219. Shah, R. D., Gonzales, F., Golez, E., Augustin, D., Caudillo, S., Abbott, A., Morello, J., Mcdonough, P. M., Paolini, P. J., and Shubeita, H. E. (2005) The Antidiabetic Agent Rosiglitazone Upregulates SERCA2 and Enhances Tnf-A- and LPS-Induced NF-κB-Dependent Transcription and Tnf-A-Induced IL-6 Secretion In Ventricular Myocytes. *Cellular Physiology and Biochemistry* **15**, 041-050
220. Moriscot, A. S., Sayen, M. R., Hartong, R., Wu, P., and Dillmann, W. H. (1997) Transcription of the Rat Sarcoplasmic Reticulum Ca2+ Adenosine Triphosphatase Gene Is Increased by 3, 5, 3′-Triiodothyronine Receptor Isoform-Specific Interactions With the Myocyte-Specific Enhancer Factor-2a 1. *Endocrinology* **138**, 26-32
221. Simonides, W., Thelen, M., Van Der Linden, C., Muller, A., and Van Hardeveld, C. (2001) Mechanism of Thyroid-Hormone Regulated Expression of the SERCA Genes In Skeletal Muscle: Implications For Thermogenesis. *Bioscience Reports* **21**, 139-154

222. Misquitta, C. M., Mack, D. P., and Grover, A. K. (1999) Sarco/Endoplasmic Reticulum Ca2+(SERCA)-Pumps: Link To Heart Beats and Calcium Waves. *Cell Calcium* **25**, 277-290
223. Bupha-Intr, T., and Wattanapermpool, J. (2006) Regulatory Role of Ovarian Sex Hormones In Calcium Uptake Activity of Cardiac Sarcoplasmic Reticulum. *American Journal of Physiology - Heart and Circulatory Physiology* **291**, H1101-H1108
224. Dash, R., Frank, K. F., Carr, A. N., Moravec, C. S., and Kranias, E. G. (2001) Gender Influences On Sarcoplasmic Reticulum Ca2+-Handling In Failing Human Myocardium. *Journal of Molecular and Cellular Cardiology* **33**, 1345-1353
225. Hughes, P. J., Mclellan, H., Lowes, D. A., Kahn, S. Z., Bilmen, J. G., Tovey, S. C., Godfrey, R. E., Michell, R. H., Kirk, C. J., and Michelangeli, F. (2000) Estrogenic Alkylphenols Induce Cell Death by Inhibiting Testis Endoplasmic Reticulum Ca2+ Pumps. *Biochemical and Biophysical Research Communications* **277**, 568-574
226. Muldrew, E., and Brent, J. (2008) Estrogen Increases The Expression of Intracellular Calcium Uptake Mechanisms In Coronary Arteries. *The Faseb Journal* **22**, 1181.1189
227. Kulkarni, R. N., Roper, M. G., Dahlgren, G., Shih, D. Q., Kauri, L. M., Peters, J. L., Stoffel, M., and Kennedy, R. T. (2004) Islet Secretory Defect In Insulin Receptor Substrate 1 Null Mice Is Linked With Reduced Calcium Signaling and Expression of Sarco(Endo)Plasmic Reticulum Ca2+-ATPase (SERCA)-2b and -3. *Diabetes* **53**, 1517-1525
228. Xu, G. G., Gao, Z.-Y., Borge, P. D., Jegier, P. A., Young, R. A., and Wolf, B. A. (2000) Insulin Regulation of β-Cell Function Involves a Feedback Loop On SERCA Gene Expression, Ca2+ Homeostasis, and Insulin Expression and Secretion. *Biochemistry* **39**, 14912-14919
229. Toyofuku, T., Kurzydlowski, K., Narayanan, N., and Maclennan, D. H. (1994) Identification of Ser38 as the Site In Cardiac Sarcoplasmic Reticulum Ca2+-ATPase That Is Phosphorylated by Ca2+/Calmodulin-Dependent Protein Kinase. *Journal of Biological Chemistry* **269**, 26492-26496
230. Dadi, P. K., Vierra, N. C., Ustione, A., Piston, D. W., Colbran, R. J., and Jacobson, D. A. (2014) Inhibition of Pancreatic Beta-Cell CAMKII Reduces Glucose-Stimulated Calcium Influx and Insulin Secretion, Impairing Glucose Tolerance. *Journal of Biological Chemistry*
231. Frank, K. F., Bolck, B., Erdmann, E., and Schwinger, R. H. (2003) Sarcoplasmic Reticulum Ca2+-ATPase Modulates Cardiac Contraction and Relaxation. *Cardiovascular Research* **57**, 20-27
232. Ashcroft, S. J. (1994) Protein Phosphorylation and Beta-Cell Function. *Diabetologia* **37 Suppl 2**, S21-29
233. Martinez-Ruiz, A., and Lamas, S. (2007) Signalling by NO-Induced Protein S-Nitrosylation and S-Glutathionylation: Convergences and Divergences. *Cardiovascular Research* **75**, 220-228
234. Peluffo, G., and Radi, R. (2007) Biochemistry of Protein Tyrosine Nitration In Cardiovascular Pathology. *Cardiovascular Research* **75**, 291-302
235. Grover, A. K., and Samson, S. E. (1997) Peroxide Resistance of ER Ca2+ Pump In Endothelium: Implications to Coronary Artery Function. *American Journal of Physiology - Cell Physiology* **273**, C1250-C1258

236. Sharov, V. S., Dremina, E. S., Galeva, N. A., Williams, T. D., and Schoneich, C. (2006) Quantitative Mapping of Oxidation-Sensitive Cysteine Residues In SERCA In Vivo and In Vitro by HPLC-Electrospray-Tandem MS: Selective Protein Oxidation During Biological Aging. *The Biochemical Journal* **394**, 605-615
237. Morla, M., Iglesias, A., Sauleda, J., Cosio, B., Agusti, A., and Busquets, X. (2007) [Reduced Expression of the Sarcoplasmic Calcium Pump SERCA2 In Skeletal Muscle from Patients With Chronic Obstructive Pulmonary Disease and Low Body Weight]. *Archivos De Bronconeumologia* **43**, 4-8
238. Bigelow, D. J. (2009) Nitrotyrosine-Modified SERCA2: A Cellular Sensor of Reactive Nitrogen Species. *Pflugers Archiv : European Journal of Physiology* **457**, 701-710
239. Viner, R. I., Ferrington, D. A., Williams, T. D., Bigelow, D. J., and Schoneich, C. (1999) Protein Modification During Biological Aging: Selective Tyrosine Nitration of the SERCA2a Isoform of the Sarcoplasmic Reticulum Ca2+-ATPase In Skeletal Muscle. *The Biochemical Journal* **340 (Pt 3)**, 657-669
240. Lancel, S., Qin, F., Lennon, S. L., Zhang, J., Tong, X., Mazzini, M. J., Kang, Y. J., Siwik, D. A., Cohen, R. A., and Colucci, W. S. (2010) Short Communication: Oxidative Posttranslational Modifications Mediate Decreased SERCA Activity and Myocyte Dysfunction In Gαq-Overexpressing Mice. *Circulation Research* **107**, 228-232
241. Adachi, T., Weisbrod, R. M., Pimentel, D. R., Ying, J., Sharov, V. S., Schoneich, C., and Cohen, R. A. (2004) S-Glutathiolation by Peroxynitrite Activates SERCA During Arterial Relaxation by Nitric Oxide. *Nature Medicine* **10**, 1200-1207
242. Cohen, R. A., and Adachi, T. (2006) Nitric-Oxide-Induced Vasodilatation: Regulation by Physiologic S-Glutathiolation and Pathologic Oxidation of the Sarcoplasmic Endoplasmic Reticulum Calcium ATPase. *Trends In Cardiovascular Medicine* **16**, 109-114
243. Yan, Y., Wei, C.-L., Zhang, W.-R., Cheng, H.-P., and Liu, J. (2006) Cross-Talk Between Calcium and Reactive Oxygen Species Signaling. *Acta Pharmacologica Sinica* **27**, 821-826
244. Grover, A. K., Kwan, C. Y., and Samson, S. E. (2003) Effects of Peroxynitrite On Sarco/Endoplasmic Reticulum Ca2+ Pump Isoforms SERCA2b and SERCA3a. *American Journal of Physiology. Cell Physiology* **285**, C1537-1543
245. Mengesdorf, T., Althausen, S., Oberndorfer, I., and Paschen, W. (2001) Response of Neurons to an Irreversible Inhibition of Endoplasmic Reticulum Ca(2+)-ATPase: Relationship Between Global Protein Synthesis and Expression and Translation of Individual Genes. *The Biochemical Journal* **356**, 805-812
246. Periasamy, M., and Kalyanasundaram, A. (2007) SERCA Pump Isoforms: Their Role In Calcium Transport and Disease. *Muscle & Nerve* **35**, 430-442
247. Babu, D. A., Deering, T. G., and Mirmira, R. G. (2007) A Feat of Metabolic Proportions: Pdx1 Orchestrates Islet Development and Function In the Maintenance of Glucose Homeostasis. *Molecular Genetics and Metabolism* **92**, 43-55
248. Mckinnon, C. M., and Docherty, K. (2001) Pancreatic Duodenal Homeobox-1, Pdx-1, a Major Regulator of Beta Cell Identity and Function. *Diabetologia* **44**, 1203-1214
249. Offield, M. F., Jetton, T. L., Labosky, P. A., Ray, M., Stein, R. W., Magnuson, M. A., Hogan, B. L., and Wright, C. V. (1996) Pdx-1 Is Required For Pancreatic Outgrowth and Differentiation of the Rostral Duodenum. *Development* **122**, 983-995

250. Sander, M., Sussel, L., Conners, J., Scheel, D., Kalamaras, J., Dela Cruz, F., Schwitzgebel, V., Hayes-Jordan, A., and German, M. (2000) Homeobox Gene Nkx6.1 Lies Downstream of Nkx2.2 In the Major Pathway of Beta-Cell Formation In the Pancreas. *Development* **127**, 5533-5540
251. Moibi, J. A., Gupta, D., Jetton, T. L., Peshavaria, M., Desai, R., and Leahy, J. L. (2007) Peroxisome Proliferator-Activated Receptor-Gamma Regulates Expression of Pdx-1 and Nkx6.1 In INS-1 Cells. *Diabetes* **56**, 88-95
252. Zhang, X., Sun, N., Wang, L., Guo, H., Guan, Q., Cui, B., Tian, L., Gao, L., and Zhao, J. (2009) AMP-Activated Protein Kinase and Pancreatic/Duodenal Homeobox-1 Involved In Insulin Secretion Under High Leucine Exposure In Rat Insulinoma Beta-Cells. *Journal of Cellular and Molecular Medicine* **13**, 758-770
253. Jonsson, J., Ahlgren, U., Edlund, T., and Edlund, H. (1995) Ipf1, a Homeodomain Protein With A Dual Function In Pancreas Development. *The International Journal of Developmental Biology* **39**, 789-798
254. Ohlsson, H., Karlsson, O., and Edlund, T. (1988) A Beta-Cell-Specific Protein Binds to the Two Major Regulatory Sequences of the Insulin Gene Enhancer. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 4228-4231
255. Whitney, M. L., Jefferson, L. S., and Kimball, S. R. (2009) Atf4 Is Necessary and Sufficient for ER Stress-Induced Upregulation of Redd1 Expression. *Biochemical and Biophysical Research Communications* **379**, 451-455
256. Zatyka, M., Ricketts, C., Da Silva Xavier, G., Minton, J., Fenton, S., Hofmann-Thiel, S., Rutter, G. A., and Barrett, T. G. (2008) Sodium-Potassium ATPase 1 Subunit Is a Molecular Partner of Wolframin, an Endoplasmic Reticulum Protein Involved In ER Stress. *Human Molecular Genetics* **17**, 190-200
257. Gauthier, B. R., Wiederkehr, A., Baquié, M., Dai, C., Powers, A. C., Kerr-Conte, J., Pattou, F., Macdonald, R. J., Ferrer, J., and Wollheim, C. B. (2009) Pdx1 Deficiency Causes Mitochondrial Dysfunction and Defective Insulin Secretion Through TFAM Suppression. *Cell Metabolism* **10**, 110-118
258. Waeber, G., Thompson, N., Nicod, P., and Bonny, C. (1996) Transcriptional Activation of the GLUT2 Gene by The Ipf-1/Stf-1/Idx-1 Homeobox Factor. *Mol Endocrinol* **10**, 1327-1334
259. Murtaugh, L. C. (2007) Pancreas and Beta-Cell Development: from the Actual to the Possible. *Development* **134**, 427-438
260. Thomas, I. H., Saini, N. K., Adhikari, A., Lee, J. M., Kasa-Vubu, J. Z., Vazquez, D. M., Menon, R. K., Chen, M., and Fajans, S. S. (2009) Neonatal Diabetes Mellitus With Pancreatic Agenesis In an Infant With Homozygous Ipf-1 PRO63FSX60 Mutation. *Pediatric Diabetes* **10**, 492-496
261. Stoffers, D. A., Zinkin, N. T., Stanojevic, V., Clarke, W. L., and Habener, J. F. (1997) Pancreatic Agenesis Attributable to a Single Nucleotide Deletion In the Human Ipf1 Gene Coding Sequence. *Nature Genetics* **15**, 106-110
262. Kim, S. K., Selleri, L., Lee, J. S., Zhang, A. Y., Gu, X., Jacobs, Y., and Cleary, M. L. (2002) Pbx1 Inactivation Disrupts Pancreas Development and In Ipf1-Deficient Mice Promotes Diabetes Mellitus. *Nature Genetics* **30**, 430-435
263. Heller, R. S., Stoffers, D. A., Bock, T., Svenstrup, K., Jensen, J., Horn, T., Miller, C. P., Habener, J. F., Madsen, O. D., and Serup, P. (2001) Improved Glucose Tolerance and Acinar Dysmorphogenesis by Targeted Expression of Transcription Factor Pdx-1 to the Exocrine Pancreas. *Diabetes* **50**, 1553-1561

264. Schwitzgebel, V. M., Mamin, A., Brun, T., Ritz-Laser, B., Zaiko, M., Maret, A., Jornayvaz, F. R., Theintz, G. E., Michielin, O., Melloul, D., and Philippe, J. (2003) Agenesis of Human Pancreas Due To Decreased Half-Life of Insulin Promoter Factor 1. *The Journal of Clinical Endocrinology and Metabolism* **88**, 4398-4406
265. Fajans, S. S., Bell, G. I., and Polonsky, K. S. (2001) Molecular Mechanisms and Clinical Pathophysiology of Maturity-Onset Diabetes of the Young. *New England Journal of Medicine* **345**, 971-980
266. Raum, J. C., Gerrish, K., Artner, I., Henderson, E., Guo, M., Sussel, L., Schisler, J. C., Newgard, C. B., and Stein, R. (2006) Foxa2, Nkx2.2, and Pdx-1 Regulate Islet β-Cell-Specific Mafa Expression Through Conserved Sequences Located Between Base Pairs −8118 and −7750 Upstream from the Transcription Start Site. *Molecular and Cellular Biology* **26**, 5735-5743
267. Kaneto, H., Miyatsuka, T., Kawamori, D., Yamamoto, K., Kato, K., Shiraiwa, T., Katakami, N., Yamasaki, Y., Matsuhisa, M., and Matsuoka, T. A. (2008) Pdx-1 and Mafa Play a Crucial Role In Pancreatic Beta-Cell Differentiation and Maintenance of Mature Beta-Cell Function. *Endocrine Journal* **55**, 235-252
268. Watada, H., Mirmira, R. G., Leung, J., and German, M. S. (2000) Transcriptional and Translational Regulation of Beta-Cell Differentiation Factor Nkx6.1. *The Journal of Biological Chemistry* **275**, 34224-34230
269. Khoo, C., Yang, J., Weinrott, S. A., Kaestner, K. H., Naji, A., Schug, J., and Stoffers, D. A. (2012) Research Resource: the Pdx1 Cistrome of Pancreatic Islets. *Mol Endocrinol* **26**, 521-533
270. Gannon, M., Ables, E. T., Crawford, L., Lowe, D., Offield, M. F., Magnuson, M. A., and Wright, C. V. (2008) Pdx-1 Function Is Specifically Required In Embryonic Beta Cells to Generate Appropriate Numbers of Endocrine Cell Types and Maintain Glucose Homeostasis. *Developmental Biology* **314**, 406-417
271. Kulkarni, R. N., Jhala, U. S., Winnay, J. N., Krajewski, S., Montminy, M., and Kahn, C. R. (2004) Pdx-1 Haploinsufficiency Limits the Compensatory Islet Hyperplasia That Occurs In Response to Insulin Resistance. *The Journal of Clinical Investigation* **114**, 828-836
272. Buteau, J., Roduit, R., Susini, S., and Prentki, M. (1999) Glucagon-Like Peptide-1 Promotes DNA Synthesis, Activates Phosphatidylinositol 3-Kinase and Increases Transcription Factor Pancreatic and Duodenal Homeobox Gene 1 (Pdx-1) DNA Binding Activity In Beta (INS-1)-Cells. *Diabetologia* **42**, 856-864
273. Hagman, D. K., Hays, L. B., Parazzoli, S. D., and Poitout, V. (2005) Palmitate Inhibits Insulin Gene Expression by Altering Pdx-1 Nuclear Localization and Reducing Mafa Expression In Isolated Rat Islets of Langerhans. *Journal of Biological Chemistry* **280**, 32413-32418
274. Campbell, S. C., and Macfarlane, W. M. (2002) Regulation of the Pdx1 Gene Promoter In Pancreatic Beta-Cells. *Biochemical and Biophysical Research Communications* **299**, 277-284
275. Gerrish, K., Gannon, M., Shih, D., Henderson, E., Stoffel, M., Wright, C. V., and Stein, R. (2000) Pancreatic Beta Cell-Specific Transcription of the Pdx-1 Gene. The Role of Conserved Upstream Control Regions and Their Hepatic Nuclear Factor 3beta Sites. *The Journal of Biological Chemistry* **275**, 3485-3492
276. Wu, K. L., Gannon, M., Peshavaria, M., Offield, M. F., Henderson, E., Ray, M., Marks, A., Gamer, L. W., Wright, C. V., and Stein, R. (1997) Hepatocyte Nuclear Factor 3beta Is Involved In Pancreatic Beta-Cell-Specific Transcription of the Pdx-1 Gene. *Molecular and Cellular Biology* **17**, 6002-6013

277. Sharma, S., Jhala, U. S., Johnson, T., Ferreri, K., Leonard, J., and Montminy, M. (1997) Hormonal Regulation of an Islet-Specific Enhancer In the Pancreatic Homeobox Gene Stf-1. *Molecular and Cellular Biology* **17**, 2598-2604
278. Sharma, S., Leonard, J., Lee, S., Chapman, H. D., Leiter, E. H., and Montminy, M. R. (1996) Pancreatic Islet Expression of the Homeobox Factor Stf-1 Relies On an E-Box Motif That Binds USF. *The Journal of Biological Chemistry* **271**, 2294-2299
279. Ben-Shushan, E., Marshak, S., Shoshkes, M., Cerasi, E., and Melloul, D. (2001) A Pancreatic Beta-Cell-Specific Enhancer In the Human Pdx-1 Gene Is Regulated by Hepatocyte Nuclear Factor 3beta (Hnf-3beta), Hnf-1alpha, and SPS Transcription Factors. *The Journal of Biological Chemistry* **276**, 17533-17540
280. Marshak, S., Benshushan, E., Shoshkes, M., Havin, L., Cerasi, E., and Melloul, D. (2000) Functional Conservation of Regulatory Elements In the Pdx-1 Gene: Pdx-1 and Hepatocyte Nuclear Factor 3β Transcription Factors Mediate β-Cell-Specific Expression. *Molecular and Cellular Biology* **20**, 7583-7590
281. Piquer, S., Barceló-Batllori, S., Julià, M., Marzo, N., Nadal, B., Guinovart, J. J., and Gomis, R. (2007) Phosphorylation Events Implicating P38 and PI3K Mediate Tungstate-Effects In MIN6 Beta Cells. *Biochemical and Biophysical Research Communications* **358**, 385-391
282. Moede, T., Leibiger, B., Pour, H. G., Berggren, P. O., and Leibiger, I. B. (1999) Identification of a Nuclear Localization Signal, RRMKWKK, In the Homeodomain Transcription Factor Pdx-1. *Febs Letters* **461**, 229-234
283. Macfarlane, W. M., Smith, S. B., James, R. F., Clifton, A. D., Doza, Y. N., Cohen, P., and Docherty, K. (1997) The P38/Reactivating Kinase Mitogen-Activated Protein Kinase Cascade Mediates the Activation of the Transcription Factor Insulin Upstream Factor 1 and Insulin Gene Transcription by High Glucose In Pancreatic Beta-Cells. *The Journal of Biological Chemistry* **272**, 20936-20944
284. Furukawa, N., Shirotani, T., Araki, E., Kaneko, K., Todaka, M., Matsumoto, K., Tsuruzoe, K., Motoshima, H., Yoshizato, K., Kishikawa, H., and Shichiri, M. (1999) Possible Involvement of Atypical Protein Kinase C (PKC) In Glucose-Sensitive Expression of the Human Insulin Gene: DNA-Binding Activity and Transcriptional Activity of Pancreatic and Duodenal Homeobox Gene-1 (Pdx-1) Are Enhanced via Calphostin C-Sensitive But Phorbol 12-Myristate 13-Acetate (PMA) and GO-6976-Insensitive Pathway. *Endocrine Journal* **46**, 43-58
285. Szabat, M., Johnson, J. D., and Piret, J. M. (2010) Reciprocal Modulation of Adult Beta Cell Maturity by Activin A and Follistatin. *Diabetologia* **53**, 1680-1689
286. Macfarlane, W. M., Mckinnon, C. M., Felton-Edkins, Z. A., Cragg, H., James, R. F. L., and Docherty, K. (1999) Glucose Stimulates Translocation of the Homeodomain Transcription Factor Pdx1 From The Cytoplasm to the Nucleus In Pancreatic B-Cells. *Journal of Biological Chemistry* **274**, 1011-1016
287. Chun, S. Y., Mack, D. L., Moorefield, E., Oh, S. H., Kwon, T. G., Pettenati, M. J., Yoo, J. J., Coppi, P. D., Atala, A., and Soker, S. (2012) Pdx1 and Controlled Culture Conditions Induced Differentiation of Human Amniotic Fluid-Derived Stem Cells To Insulin-Producing Clusters. *Journal of Tissue Engineering and Regenerative Medicine*
288. Wu, H., Macfarlane, W. M., Tadayyon, M., Arch, J. R., James, R. F., and Docherty, K. (1999) Insulin Stimulates Pancreatic-Duodenal Homoeobox Factor-1 (Pdx1) DNA-Binding Activity and Insulin Promoter Activity In Pancreatic Beta Cells. *The Biochemical Journal* **344 Pt 3**, 813-818

289. Yoshikawa, H., Tajiri, Y., Sako, Y., Hashimoto, T., Umeda, F., and Nawata, H. (2002) Effects of Biotin On Glucotoxicity Or Lipotoxicity In Rat Pancreatic Islets. *Metabolism: Clinical and Experimental* **51**, 163-168
290. Xia, F., Dohi, T., Martin, N. M., Raskett, C. M., Liu, Q., and Altieri, D. C. (2011) Essential Role of the Small GTPase Ran In Postnatal Pancreatic Islet Development. *PLOS One* **6**, E27879
291. Kawamori, D., Kajimoto, Y., Kaneto, H., Umayahara, Y., Fujitani, Y., Miyatsuka, T., Watada, H., Leibiger, I. B., Yamasaki, Y., and Hori, M. (2003) Oxidative Stress Induces Nucleo-Cytoplasmic Translocation of Pancreatic Transcription Factor Pdx-1 Through Activation of C-Jun NH2-Terminal Kinase. *Diabetes* **52**, 2896-2904
292. Lebrun, P., Montminy, M. R., and Van Obberghen, E. (2005) Regulation of the Pancreatic Duodenal Homeobox-1 Protein by DNA-Dependent Protein Kinase. *Journal of Biological Chemistry* **280**, 38203-38210
293. Kawamori, D., Kaneto, H., Nakatani, Y., Matsuoka, T.-A., Matsuhisa, M., Hori, M., and Yamasaki, Y. (2006) The Forkhead Transcription Factor Foxo1 Bridges The Jnk Pathway and The Transcription Factor Pdx-1 Through Its Intracellular Translocation. *Journal of Biological Chemistry* **281**, 1091-1098
294. Harmon, J. S., Gleason, C. E., Tanaka, Y., Oseid, E. A., Hunter-Berger, K. K., and Robertson, R. P. (1999) In Vivo Prevention of Hyperglycemia Also Prevents Glucotoxic Effects On Pdx-1 and Insulin Gene Expression. *Diabetes* **48**, 1995-2000
295. Kaneto, H., Matsuoka, T.-A., Nakatani, Y., Kawamori, D., Miyatsuka, T., Matsuhisa, M., and Yamasaki, Y. (2005) Oxidative Stress, ER Stress, and the Jnk Pathway In Type 2 Diabetes. *Journal of Molecular Medicine* **83**, 429-439
296. Feanny, M. A., Fagan, S. P., Ballian, N., Liu, S.-H., Li, Z., Wang, X., Fisher, W., Brunicardi, F. C., and Belaguli, N. S. (2008) Pdx-1 Expression Is Associated With Islet Proliferation In Vitro and In Vivo. *Journal of Surgical Research* **144**, 8-16
297. Flier, J. S., Underhill, L. H., Polonsky, K. S., Sturis, J., and Bell, G. I. (1996) Non-Insulin-Dependent Diabetes Mellitus—A Genetically Programmed Failure of the Beta Cell to Compensate For Insulin Resistance. *New England Journal of Medicine* **334**, 777-783
298. Chang-Chen, K. J., Mullur, R., and Bernal-Mizrachi, E. (2008) β-Cell Failure as a Complication of Diabetes. *Rev Endocr Metab Disord* **9**, 329-343
299. Michalik, L., Auwerx, J., Berger, J. P., Chatterjee, V. K., Glass, C. K., Gonzalez, F. J., Grimaldi, P. A., Kadowaki, T., Lazar, M. A., O'rahilly, S., Palmer, C. N., Plutzky, J., Reddy, J. K., Spiegelman, B. M., Staels, B., and Wahli, W. (2006) International Union of Pharmacology. LXI. Peroxisome Proliferator-Activated Receptors. *Pharmacological Reviews* **58**, 726-741
300. Spiegelman, B. M. (1998) PPAR-Gamma: Adipogenic Regulator and Thiazolidinedione Receptor. *Diabetes* **47**, 507-514
301. Pettinelli, P., and Videla, L. A. (2011) Up-Regulation of PPARγ mRNA Expression In the Liver of Obese Patients: an Additional Reinforcing Lipogenic Mechanism to SREBP-1c Induction. *The Journal of Clinical Endocrinology & Metabolism* **96**, 1424-1430
302. Kersten, S., Desvergne, B., and Wahli, W. (2000) Roles of PPARs In Health and Disease. *Nature* **405**, 421-424

303. Evans-Molina, C., Robbins, R. D., Kono, T., Tersey, S. A., Vestermark, G. L., Nunemaker, C. S., Garmey, J. C., Deering, T. G., Keller, S. R., Maier, B., and Mirmira, R. G. (2009) Peroxisome Proliferator-Activated Receptor γ Activation Restores Islet Function In Diabetic Mice Through Reduction of Endoplasmic Reticulum Stress and Maintenance of Euchromatin Structure. *Molecular and Cellular Biology* **29**, 2053-2067
304. Akahoshi, T., Namai, R., Murakami, Y., Watanabe, M., Matsui, T., Nishimura, A., Kitasato, H., Kameya, T., and Kondo, H. (2003) Rapid Induction of Peroxisome Proliferator-Activated Receptor Gamma Expression In Human Monocytes by Monosodium Urate Monohydrate Crystals. *Arthritis and Rheumatism* **48**, 231-239
305. Burns, K. A., and Vanden Heuvel, J. P. (2007) Modulation of PPAR Activity via Phosphorylation. *Biochimica Et Biophysica Acta* **1771**, 952-960
306. Choi, J. H., Banks, A. S., Estall, J. L., Kajimura, S., Bostrom, P., Laznik, D., Ruas, J. L., Chalmers, M. J., Kamenecka, T. M., Bluher, M., Griffin, P. R., and Spiegelman, B. M. (2010) Anti-Diabetic Drugs Inhibit Obesity-Linked Phosphorylation of PPARgamma by Cdk5. *Nature* **466**, 451-456
307. Kim, E., Chen, F., Wang, C. C., and Harrison, L. E. (2006) Cdk5 Is a Novel Regulatory Protein In PPARgamma Ligand-Induced Antiproliferation. *International Journal of Oncology* **28**, 191-194
308. Berger, J., and Moller, D. E. (2002) The Mechanisms of Action of PPARs. *Annual Review of Medicine* **53**, 409-435
309. Issemann, I., Prince, R. A., Tugwood, J. D., and Green, S. (1993) The Retinoid X Receptor Enhances the Function of the Peroxisome Proliferator Activated Receptor. *Biochimie* **75**, 251-256
310. Nolte, R. T., Wisely, G. B., Westin, S., Cobb, J. E., Lambert, M. H., Kurokawa, R., Rosenfeld, M. G., Willson, T. M., Glass, C. K., and Milburn, M. V. (1998) Ligand Binding and Co-Activator Assembly of the Peroxisome Proliferator-Activated Receptor-Gamma. *Nature* **395**, 137-143
311. Shao, D., Rangwala, S. M., Bailey, S. T., Krakow, S. L., Reginato, M. J., and Lazar, M. A. (1998) Interdomain Communication Regulating Ligand Binding by PPAR-Gamma. *Nature* **396**, 377-380
312. Saltiel, A. R., and Kahn, C. R. (2001) Insulin Signalling and the Regulation of Glucose and Lipid Metabolism. *Nature* **414**, 799-806
313. Chinetti, G., Fruchart, J. C., and Staels, B. (2000) Peroxisome Proliferator-Activated Receptors (PPARs): Nuclear Receptors at the Crossroads Between Lipid Metabolism and Inflammation. *Inflammation Research : Official Journal of the European Histamine Research Society* **49**, 497-505
314. Dubuquoy, L., Dharancy, S., Nutten, S., Pettersson, S., Auwerx, J., and Desreumaux, P. (2002) Role of Peroxisome Proliferator-Activated Receptor Gamma and Retinoid X Receptor Heterodimer In Hepatogastroenterological Diseases. *Lancet* **360**, 1410-1418
315. Spiegelman, B. M., Puigserver, P., and Wu, Z. (2000) Regulation of Adipogenesis and Energy Balance by PPARgamma and PGC-1. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association For The Study of Obesity* **24 Suppl 4**, S8-10
316. Rosen, E. D., Kulkarni, R. N., Sarraf, P., Ozcan, U., Okada, T., Hsu, C. H., Eisenman, D., Magnuson, M. A., Gonzalez, F. J., Kahn, C. R., and Spiegelman, B. M. (2003) Targeted Elimination of Peroxisome Proliferator-Activated Receptor Gamma In Beta Cells Leads To Abnormalities In Islet Mass Without Compromising Glucose Homeostasis. *Molecular and Cellular Biology* **23**, 7222-7229

317. Li, Y., Ge, M., Ciani, L., Kuriakose, G., Westover, E. J., Dura, M., Covey, D. F., Freed, J. H., Maxfield, F. R., Lytton, J., and Tabas, I. (2004) Enrichment of Endoplasmic Reticulum With Cholesterol Inhibits Sarcoplasmic-Endoplasmic Reticulum Calcium ATPase-2b Activity In Parallel With Increased Order of Membrane Lipids: Implications For Depletion of Endoplasmic Reticulum Calcium Stores and Apoptosis In Cholesterol-Loaded Macrophages. *The Journal of Biological Chemistry* **279**, 37030-37039
318. Ubeda, M., Rukstalis, J. M., and Habener, J. F. (2006) Inhibition of Cyclin-Dependent Kinase 5 Activity Protects Pancreatic Beta Cells From Glucotoxicity. *The Journal of Biological Chemistry* **281**, 28858-28864
319. Smith, U. (2001) Pioglitazone: Mechanism of Action. *International Journal of Clinical Practice. Supplement*, 13-18
320. Gillies, P. S., and Dunn, C. J. (2000) Pioglitazone. *Drugs* **60**, 333-343; Discussion 344-335
321. Miller, J. L. (1999) FDA Approves Pioglitazone For Diabetes. *American Journal of Health-System Pharmacy: AJHP: Official Journal of the American Society of Health-System Pharmacists* **56**, 1698
322. Walter, H., and Lubben, G. (2005) Potential Role of Oral Thiazolidinedione Therapy In Preserving Beta-Cell Function In Type 2 Diabetes Mellitus. *Drugs* **65**, 1-13
323. Ishida, H., Takizawa, M., Ozawa, S., Nakamichi, Y., Yamaguchi, S., Katsuta, H., Tanaka, T., Maruyama, M., Katahira, H., Yoshimoto, K., Itagaki, E., and Nagamatsu, S. (2004) Pioglitazone Improves Insulin Secretory Capacity and Prevents the Loss of Beta-Cell Mass In Obese Diabetic db/db Mice: Possible Protection of Beta Cells From Oxidative Stress. *Metabolism: Clinical and Experimental* **53**, 488-494
324. Lebovitz, H. E., Kreider, M., and Freed, M. I. (2002) Evaluation of Liver Function In Type 2 Diabetic Patients During Clinical Trials: Evidence That Rosiglitazone Does Not Cause Hepatic Dysfunction. *Diabetes Care* **25**, 815-821
325. Home, P. D., Pocock, S. J., Beck-Nielsen, H., Gomis, R., Hanefeld, M., Dargie, H., Komajda, M., Gubb, J., Biswas, N., and Jones, N. P. (2005) Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycaemia In Diabetes (Record): Study Design and Protocol. *Diabetologia* **48**, 1726-1735
326. Stafylas, P. C., Sarafidis, P. A., and Lasaridis, A. N. (2009) The Controversial Effects of Thiazolidinediones On Cardiovascular Morbidity and Mortality. *International Journal of Cardiology* **131**, 298-304
327. Kaul, S., Bolger, A. F., Herrington, D., Giugliano, R. P., and Eckel, R. H. (2010) Thiazolidinedione Drugs and Cardiovascular Risks: a Science Advisory from the American Heart Association and American College of Cardiology Foundation. *Journal of the American College of Cardiology* **55**, 1885-1894
328. Lewis, J. D., Ferrara, A., Peng, T., Hedderson, M., Bilker, W. B., Quesenberry, C. P., Vaughn, D. J., Nessel, L., Selby, J., and Strom, B. L. (2011) Risk of Bladder Cancer Among Diabetic Patients Treated With Pioglitazone: Interim Report of a Longitudinal Cohort Study. *Diabetes Care* **34**, 916-922
329. Khalaf, K. I., and Taegtmeyer, H. (2010) Insulin Sensitizers and Heart Failure: an Engine Flooded With Fuel. *Current Hypertension Reports* **12**, 399-401
330. Kitamura, T., and Ido Kitamura, Y. (2007) Role of Foxo Proteins In Pancreatic Beta Cells. *Endocrine Journal* **54**, 507-515
331. Accili, D., and Arden, K. C. (2004) Foxos At The Crossroads of Cellular Metabolism, Differentiation, and Transformation. *Cell* **117**, 421-426

332. Kodama, S., Toyonaga, T., Kondo, T., Matsumoto, K., Tsuruzoe, K., Kawashima, J., Goto, H., Kume, K., Kume, S., Sakakida, M., and Araki, E. (2005) Enhanced Expression of Pdx-1 and Ngn3 by Exendin-4 During β Cell Regeneration In STZ-Treated Mice. *Biochemical and Biophysical Research Communications* **327**, 1170-1178
333. Nakae, J., Biggs, W. H., 3rd, Kitamura, T., Cavenee, W. K., Wright, C. V., Arden, K. C., and Accili, D. (2002) Regulation of Insulin Action and Pancreatic Beta-Cell Function by Mutated Alleles of the Gene Encoding Forkhead Transcription Factor Foxo1. *Nature Genetics* **32**, 245-253
334. Kitamura, Y. I., Kitamura, T., Kruse, J.-P., Raum, J. C., Stein, R., Gu, W., and Accili, D. (2005) Foxo1 Protects Against Pancreatic β Cell Failure Through NeuroD and Mafa Induction. *Cell Metabolism* **2**, 153-163
335. Gupta, D., Leahy, A. A., Monga, N., Peshavaria, M., Jetton, T. L., and Leahy, J. L. (2013) PPARγ and Its Target Genes Are Downstream Effectors of Foxo1 In Islet Beta-Cells: Mechanism of Beta-Cell Compensation and Failure. *Journal of Biological Chemistry*
336. Kim, S.-J., Winter, K., Nian, C., Tsuneoka, M., Koda, Y., and Mcintosh, C. H. S. (2005) Glucose-Dependent Insulinotropic Polypeptide (GIP) Stimulation of Pancreatic β-Cell Survival Is Dependent Upon Phosphatidylinositol 3-Kinase (PI3K)/Protein Kinase B (PKB) Signaling, Inactivation of the Forkhead Transcription Factor Foxo1, and Down-Regulation of Bax Expression. *Journal of Biological Chemistry* **280**, 22297-22307
337. Qiu, Y., Guo, M., Huang, S., and Stein, R. (2002) Insulin Gene Transcription Is Mediated by Interactions Between the P300 Coactivator and Pdx-1, Beta2, and E47. *Molecular and Cellular Biology* **22**, 412-420
338. Fernandez-Zapico, M. E., Van Velkinburgh, J. C., Gutiérrez-Aguilar, R., Neve, B., Froguel, P., Urrutia, R., and Stein, R. (2009) MODY7 Gene, Klf11, Is a Novel P300-Dependent Regulator of Pdx-1 (MODY4) Transcription In Pancreatic Islet β Cells. *Journal of Biological Chemistry* **284**, 36482-36490
339. Asfari, M., Janjic, D., Meda, P., Li, G., Halban, P. A., and Wollheim, C. B. (1992) Establishment of 2-Mercaptoethanol-Dependent Differentiated Insulin-Secreting Cell Lines. *Endocrinology* **130**, 167-178
340. Wang, H., Iezzi, M., Theander, S., Antinozzi, P. A., Gauthier, B. R., Halban, P. A., and Wollheim, C. B. (2005) Suppression of Pdx-1 Perturbs Proinsulin Processing, Insulin Secretion and GLP-1 Signalling In INS-1 Cells. *Diabetologia* **48**, 720-731
341. Ravier, M. A., Daro, D., Roma, L. P., Jonas, J.-C., Cheng-Xue, R., Schuit, F. C., and Gilon, P. (2011) Mechanisms of Control of the Free Ca2+ Concentration In The Endoplasmic Reticulum of Mouse Pancreatic β-Cells: Interplay With Cell Metabolism, Ca2+ and the Role of SERCA2b and SERCA3. *Diabetes* **60**, 2533-2545
342. Iype, T., Francis, J., Garmey, J. C., Schisler, J. C., Nesher, R., Weir, G. C., Becker, T. C., Newgard, C. B., Griffen, S. C., and Mirmira, R. G. (2005) Mechanism of Insulin Gene Regulation by the Pancreatic Transcription Factor Pdx-1: Application of Pre-mRNA Analysis and Chromatin Immunoprecipitation to Assess Formation of Functional Transcriptional Complexes. *The Journal of Biological Chemistry* **280**, 16798-16807
343. Docherty, H. M., Hay, C. W., Ferguson, L. A., Barrow, J., Durward, E., and Docherty, K. (2005) Relative Contribution of Pdx-1, Mafa and E47/Beta2 to the Regulation of the Human Insulin Promoter. *The Biochemical Journal* **389**, 813-820

344. Stoffers, D. A., Zinkin, N. T., Stanojevic, V., Clarke, W. L., and Habener, J. F. (1997) Pancreatic Agenesis Attributable to a Single Nucleotide Deletion In the Human Ipf1 Gene Coding Sequence. *Nature Genetics* **15**, 106-110
345. Brissova, M., Shiota, M., Nicholson, W. E., Gannon, M., Knobel, S. M., Piston, D. W., Wright, C. V., and Powers, A. C. (2002) Reduction In Pancreatic Transcription Factor Pdx-1 Impairs Glucose-Stimulated Insulin Secretion. *The Journal of Biological Chemistry* **277**, 11225-11232
346. Johnson, J. D., Ahmed, N. T., Luciani, D. S., Han, Z., Tran, H., Fujita, J., Misler, S., Edlund, H., and Polonsky, K. S. (2003) Increased Islet Apoptosis In Pdx1+/- Mice. *The Journal of Clinical Investigation* **111**, 1147-1160
347. Scheuner, D., and Kaufman, R. J. (2008) The Unfolded Protein Response: a Pathway That Links Insulin Demand With Beta-Cell Failure and Diabetes. *Endocrine Reviews* **29**, 317-333
348. Ozcan, U., Cao, Q., Yilmaz, E., Lee, A. H., Iwakoshi, N. N., Ozdelen, E., Tuncman, G., Gorgun, C., Glimcher, L. H., and Hotamisligil, G. S. (2004) Endoplasmic Reticulum Stress Links Obesity, Insulin Action, and Type 2 Diabetes. *Science* **306**, 457-461
349. Araki, E., Oyadomari, S., and Mori, M. (2003) Impact of Endoplasmic Reticulum Stress Pathway On Pancreatic β-Cells and Diabetes Mellitus. *Exp. Biol. Med.* **228**, 1213-1217
350. Tersey, S. A., Nishiki, Y., Templin, A. T., Cabrera, S. M., Stull, N. D., Colvin, S. C., Evans-Molina, C., Rickus, J. L., Maier, B., and Mirmira, R. G. (2012) Islet β-Cell Endoplasmic Reticulum Stress Precedes the Onset of Type 1 Diabetes In the Nonobese Diabetic Mouse Model. *Diabetes* **61**, 818-827
351. Eizirik, D. L., Miani, M., and Cardozo, A. K. (2013) Signalling Danger: Endoplasmic Reticulum Stress and the Unfolded Protein Response In Pancreatic Islet Inflammation. *Diabetologia* **56**, 234-241
352. Coe, H., and Michalak, M. (2009) Calcium Binding Chaperones of the Endoplasmic Reticulum. *General Physiology and Biophysics* **28 Spec No Focus**, F96-F103
353. Baggio, L. L., and Drucker, D. J. (2007) Biology of Incretins: GLP-1 and GIP. *Gastroenterology* **132**, 2131-2157
354. Gautier, J. F., Choukem, S. P., and Girard, J. (2008) Physiology of Incretins (GIP and GLP-1) and Abnormalities In Type 2 Diabetes. *Diabetes & Metabolism* **34, Supplement 2**, S65-S72
355. Shennan, K. I., Taylor, N. A., and Docherty, K. (1994) Calcium- and pH-Dependent Aggregation and Membrane Association of the Precursor of the Pro-hormone Convertase PC2. *Journal of Biological Chemistry* **269**, 18646-18650
356. Seidah, N. G. (2011) What Lies Ahead For the Proprotein Convertases? *Ann. N. Y. Acad. Sci.* **1220**, 149-161
357. Kono, T., Ahn, G., Moss, D. R., Gann, L., Zarain-Herzberg, A., Nishiki, Y., Fueger, P. T., Ogihara, T., and Evans-Molina, C. (2012) PPAR-γ Activation Restores Pancreatic Islet SERCA2 Levels and Prevents β-Cell Dysfunction Under Conditions of Hyperglycemic and Cytokine Stress. *Molecular Endocrinology* **26**, 257-271
358. Khoo, C., Yang, J., Rajpal, G., Wang, Y., Liu, J., Arvan, P., and Stoffers, D. A. (2011) Endoplasmic Reticulum Oxidoreductin-1-Like Beta (ERO1lbeta) Regulates Susceptibility to Endoplasmic Reticulum Stress and Is Induced by Insulin Flux In Beta-Cells. *Endocrinology* **152**, 2599-2608

359. Chu, K., and Tsai, M.-J. (2005) Neuronatin, a Downstream Target of Beta2/NeuroD1 In the Pancreas, Is Involved In Glucose-Mediated Insulin Secretion. *Diabetes* **54**, 1064-1073
360. Hatanaka, M., Tanabe, K., Yanai, A., Ohta, Y., Kondo, M., Akiyama, M., Shinoda, K., Oka, Y., and Tanizawa, Y. (2011) Wolfram Syndrome 1 Gene (Wfs1) Product Localizes to Secretory Granules and Determines Granule Acidification In Pancreatic β-Cells. *Hum. Mol. Genet.* **20**, 1274-1284
361. Fonseca, S. G., Urano, F., Weir, G. C., Gromada, J., and Burcin, M. (2012) Wolfram Syndrome 1 and Adenylyl Cyclase 8 Interact at the Plasma Membrane to Regulate Insulin Production and Secretion. *Nature Cell Biology* **14**, 1105-1112
362. Inoue, H., Tanizawa, Y., Wasson, J., Behn, P., Kalidas, K., Bernal-Mizrachi, E., Mueckler, M., Marshall, H., Donis-Keller, H., and Crock, P. (1998) A Gene Encoding a Transmembrane Protein Is Mutated In Patients With Diabetes Mellitus and Optic Atrophy (Wolfram Syndrome). *Nat. Genet.* **20**, 143-148
363. Barrett, T. G., and Bundey, S. E. (1997) Wolfram (DIDMOAD) Syndrome. *J. Med. Genet.* **34**, 838-841
364. Sandhu, M. S., Weedon, M. N., Fawcett, K. A., Wasson, J., Debenham, S. L., Daly, A., Lango, H., Frayling, T. M., Neumann, R. J., Sherva, R., Blech, I., Pharoah, P. D., Palmer, C. N., Kimber, C., Tavendale, R., Morris, A. D., Mccarthy, M. I., Walker, M., Hitman, G., Glaser, B., Permutt, M. A., Hattersley, A. T., Wareham, N. J., and Barroso, I. (2007) Common Variants In Wfs1 Confer Risk of Type 2 Diabetes. *Nature Genetics* **39**, 951-953
365. Riggs, A. C., Bernal-Mizrachi, E., Ohsugi, M., Wasson, J., Fatrai, S., Welling, C., Murray, J., Schmidt, R. E., Herrera, P. L., and Permutt, M. A. (2005) Mice Conditionally Lacking the Wolfram Gene In Pancreatic Islet Beta Cells Exhibit Diabetes as a Result of Enhanced Endoplasmic Reticulum Stress and Apoptosis. *Diabetologia* **48**, 2313-2321
366. Ishihara, H., Takeda, S., Tamura, A., Takahashi, R., Yamaguchi, S., Takei, D., Yamada, T., Inoue, H., Soga, H., Katagiri, H., Tanizawa, Y., and Oka, Y. (2004) Disruption of the Wfs1 Gene In Mice Causes Progressive β-Cell Loss and Impaired Stimulus–Secretion Coupling In Insulin Secretion. *Hum. Mol. Genet.* **13**, 1159-1170
367. Takei, D., Ishihara, H., Yamaguchi, S., Yamada, T., Tamura, A., Katagiri, H., Maruyama, Y., and Oka, Y. (2006) Wfs1 Protein Modulates the Free Ca2+ Concentration In the Endoplasmic Reticulum. *FEBS Lett.* **580**, 5635-5640
368. Wu, Z., Xie, Y., Bucher, N., and Farmer, S. R. (1995) Conditional Ectopic Expression of C/EBP Beta In NIH-3T3 Cells Induces PPAR Gamma and Stimulates Adipogenesis. *Genes & Development* **9**, 2350-2363
369. Jackson Laboratories, T. Mice Database, Mouse Strain B6.129-Ppargtm2rev/J. *N.D. Web* **Apr. 25**, 2014
370. Yuan, Y., Hartland, K., Boskovic, Z., Wang, Y., Walpita, D., Lysy, Philippe A., Zhong, C., Young, Damian W., Kim, Y.-K., Tolliday, Nicola J., Sokal, Etienne M., Schreiber, Stuart L., and Wagner, Bridget K. (2013) A Small-Molecule Inducer of Pdx1 Expression Identified by High-Throughput Screening. *Chemistry & Biology* **20**, 1513-1522
371. Zarain-Herzberg, A., Afzal, N., Elimban, V., and Dhalla, N. S. (1996) Decreased Expression of Cardiac Sarcoplasmic Reticulum Ca(2+)-Pump ATPase In Congestive Heart Failure Due to Myocardial Infarction. *Molecular and Cellular Biochemistry* **163-164**, 285-290

372. Gómez, A. M., Guatimosim, S., Dilly, K. W., Vassort, G., and Lederer, W. J. (2001) Heart Failure After Myocardial Infarction: Altered Excitation-Contraction Coupling. *Circulation* **104**, 688-693
373. Shah, S. J., Blair, J. E. A., Filippatos, G. S., Macarie, C., Ruzyllo, W., Korewicki, J., Bubenek-Turconi, S. I., Ceracchi, M., Bianchetti, M., Carminati, P., Kremastinos, D., Grzybowski, J., Valentini, G., Sabbah, H. N., and Gheorghiade, M. (2009) Effects of Istaroxime On Diastolic Stiffness In Acute Heart Failure Syndromes: Results From the Hemodynamic, Echocardiographic, and Neurohormonal Effects of Istaroxime, a Novel Intravenous Inotropic and Lusitropic Agent: a Randomized Controlled Trial In Patients Hospitalized With Heart Failure (HORIZON-HF) Trial. *American Heart Journal* **157**, 1035-1041
374. Yu, Y.-L., Chou, R.-H., Chen, L.-T., Shyu, W.-C., Hsieh, S.-C., Wu, C.-S., Zeng, H.-J., Yeh, S.-P., Yang, D.-M., Hung, S.-C., and Hung, M.-C. (2011) EZH2 Regulates Neuronal Differentiation of Mesenchymal Stem Cells Through PIP5K1c-Dependent Calcium Signaling. *Journal of Biological Chemistry* **286**, 9657-9667
375. Yi, M., Weaver, D., Eisner, V., Várnai, P., Hunyady, L., Ma, J., Csordás, G., and Hajnóczky, G. (2012) Switch From ER-Mitochondrial To SR-Mitochondrial Calcium Coupling During Muscle Differentiation. *Cell Calcium* **52**, 355-365
376. Barradas, A. M. C., Fernandes, H. A. M., Groen, N., Chai, Y. C., Schrooten, J., Van De Peppel, J., Van Leeuwen, J. P. T. M., Van Blitterswijk, C. A., and De Boer, J. (2012) A Calcium-Induced Signaling Cascade Leading to Osteogenic Differentiation of Human Bone Marrow-Derived Mesenchymal Stromal Cells. *Biomaterials* **33**, 3205-3215
377. Animals, C. F. T. U. O. T. G. F. T. C. A. U. O. L. (2011) *Guide For The Care and Use of Laboratory Animals*, 8th Ed., National Academies Press, Washington, D.C., U.S.A.
378. Chaudhry, Z. Z., Morris, D. L., Moss, D. R., Sims, E. K., Chiong, Y., Kono, T., and Evans-Molina, C. (2013) Streptozotocin Is Equally Diabetogenic Whether Administered to Fed or Fasted Mice. *Laboratory Animals* **47**, 257-265
379. Sims, E. K., Hatanaka, M., Morris, D. L., Tersey, S. A., Kono, T., Chaudry, Z. Z., Day, K. H., Moss, D. R., Stull, N. D., Mirmira, R. G., and Evans-Molina, C. (2013) Divergent Compensatory Responses to High-Fat Diet Between C57BL6/J and C57BLKS/J Inbred Mouse Strains. *American Journal of Physiology. Endocrinology and Metabolism* **305**, E1495-1511
380. Ogihara, T., Chuang, J. C., Vestermark, G. L., Garmey, J. C., Ketchum, R. J., Huang, X., Brayman, K. L., Thorner, M. O., Repa, J. J., Mirmira, R. G., and Evans-Molina, C. (2010) Liver X Receptor Agonists Augment Human Islet Function Through Activation of Anaplerotic Pathways and Glycerolipid/Free Fatty Acid Cycling. *The Journal of Biological Chemistry* **285**, 5392-5404
381. Fisher, M. M., Perez Chumbiauca, C. N., Mather, K. J., Mirmira, R. G., and Tersey, S. A. (2013) Detection of Islet β-Cell Death In Vivo by Multiplex PCR Analysis of Differentially Methylated DNA. *Endocrinology* **154**, 3476-3481
382. Park, S. W., Zhou, Y., Lee, J., and Ozcan, U. (2010) Sarco(Endo)Plasmic Reticulum Ca2+-ATPase 2b Is a Major Regulator of Endoplasmic Reticulum Stress and Glucose Homeostasis In Obesity. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 19320-19325
383. Bain, J. R., Schisler, J. C., Takeuchi, K., Newgard, C. B., and Becker, T. C. (2004) An Adenovirus Vector for Efficient RNA Interference-Mediated Suppression of Target Genes In Insulinoma Cells and Pancreatic Islets of Langerhans. *Diabetes* **53**, 2190-2194

384. Gotoh, M., Maki, T., Kiyoizumi, T., Satomi, S., and Monaco, A. P. (1985) An Improved Method for Isolation of Mouse Pancreatic Islets. *Transplantation* **40**, 437-438
385. Sturek, J. M., Castle, J. D., Trace, A. P., Page, L. C., Castle, A. M., Evans-Molina, C., Parks, J. S., Mirmira, R. G., and Hedrick, C. C. (2010) An Intracellular Role For ABCG1-Mediated Cholesterol Transport In the Regulated Secretory Pathway of Mouse Pancreatic Beta Cells. *The Journal of Clinical Investigation* **120**, 2575-2589
386. Evans-Molina, C., Garmey, J. C., Ketchum, R., Brayman, K. L., Deng, S., and Mirmira, R. G. (2007) Glucose Regulation of Insulin Gene Transcription and Pre-mRNA Processing In Human Islets. *Diabetes* **56**, 827-835
387. Chakrabarti, S. K., James, J. C., and Mirmira, R. G. (2002) Quantitative Assessment of Gene Targeting In Vitro and In Vivo by the Pancreatic Transcription Factor, Pdx1. Importance of Chromatin Structure In Directing Promoter Binding. *The Journal of Biological Chemistry* **277**, 13286-13293
388. Lipson, K. L., Fonseca, S. G., Ishigaki, S., Nguyen, L. X., Foss, E., Bortell, R., Rossini, A. A., and Urano, F. (2006) Regulation of Insulin Biosynthesis In Pancreatic Beta Cells by an Endoplasmic Reticulum-Resident Protein Kinase IRE1. *Cell Metabolism* **4**, 245-254
389. Mccombs, J. E., and Palmer, A. E. (2008) Measuring Calcium Dynamics In Living Cells With Genetically Encodable Calcium Indicators. *Methods* **46**, 152-159
390. Palmer, A. E., Jin, C., Reed, J. C., and Tsien, R. Y. (2004) Bcl-2-Mediated Alterations In Endoplasmic Reticulum Ca2+ Analyzed With an Improved Genetically Encoded Fluorescent Sensor. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 17404-17409
391. Day, R. N. Measuring Protein Interactions Using Förster Resonance Energy Transfer and Fluorescence Lifetime Imaging Microscopy. *Methods*
392. Abdi, H. (2007) The Bonferonni and Šidák Corrections for Multiple Comparisons. *Encyclopedia of Measurement and Statistics* **3**, 103-107

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BIOL-K101 Concepts of Biology I, Recitation Leader — 2006

MENTORING
Bready, Devin, Undergraduate work study student — 2011
Kumar, Nimisha, Summer research fellowship student — 2011

CONFERENCES ATTENDED

ORAL PRESENTATIONS- REGIONAL
"Pdx-1 Regulates Transcription of SERCA2b and Ameliorates Endoplasmic Reticulum Stress in the β Cell", Johnson JS. — 2014

POSTER PRESENTATIONS-NATIONAL

"Pdx-1 loss contributes to β cell SERCA2 transcriptional dysregulation in Type 2 diabetes mellitus", Johnson JS, Kono T, Tong X, Moss D, Colvin S, Fueger PT, and Evans-Molina C.	73rd Scientific Sessions of the American Diabetes Association	2013

POSTER PRESENTATIONS-REGIONAL

"Pdx-1 loss contributes to β cell SERCA2 transcriptional dysregulation in Type 2 diabetes mellitus", Johnson JS, Kono T, Tong X, Moss D, Colvin S, Fueger PT, and Evans-Molina C.	6th Annual Midwest Islet Club	2013
"Transcriptional Role of Pdx-1 in SERCA2 Expression and Calcium Homeostasis in Pancreatic β Cells", Johnson JS, Kono T and Evans-Molina C.	1st Annual Midwest Regional Physician Scientist Program Meeting	2012
"Transcriptional Role of Pdx-1 in SERCA2 Expression and Calcium Homeostasis in Pancreatic β Cells", Johnson JS, Kono T and Evans-Molina C.	5th Annual Midwest Islet Club	2012
"Amelioration of Proximal Tubule Epithelial Detachment Due to Hypoxia Utilizing Metformin", Johnson JS and Atkinson SA	1st Annual Meeting of the Indiana Physiological Society	2011

PRINT AND/OR ELECTRONIC PUBLICATIONS

Justin S. Johnson, Tatsuyoshi Kono, Xin Tong, Wataru Yamamoto, Angel Zarain-Herzberg, Matthew J. Merrins, Leslie S. Satin, Patrick Gilon, and Carmella Evans-Molina, "Pancreatic and Duodenal Homeobox Protein 1 (Pdx-1) Maintains Endoplasmic Reticulum Calcium Levels Through Transcriptional Regulation of Sarco-endoplasmic Reticulum Calcium ATPase 2b (SERCA2b) in the Islet β Cell", *The Journal of Biological Chemistry*; under revision, 5/16/2014

Justin S. Johnson, Carmella Evans-Molina, "Translational Implications of the β-cell Epigenome in Diabetes Mellitus", *Translational Research*; in press, available online 3/12/2014